BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

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VOLUME LVI

BALTIMORE
THE JOHNS HOPKINS PRESS
1935

EXPERIMENTAL APPROACH TO THE CANCER PROBLEM[1]

I. Four Important Phases of Cancer Research

II. Avian Tumors in Relation to the General Problem of Malignancy

JAMES B. MURPHY, M.D.

From the Laboratories of the Rockefeller Institute for Medical Research

INTRODUCTION

May I express to the members of the Thayer Lecture Committee my appreciation for this opportunity to participate in an event designed to honor Dr. Thayer. In common with many of this audience I shared the privilege of having had the direct example of his distinguished and scholarly approach to clinical medicine, and of his inspiring qualities as a teacher. In addition, I owe a very personal debt of gratitude which it gives me pleasure to acknowledge on this occasion.

It is a particularly difficult subject which I have chosen to discuss with you, for the cancer problem is only in the process of formulation. Not even a possible theory exists which can reconcile the apparently conflicting facts brought out by the experimental examination of the subject, and justly due to this lack of any unity, the trends of investigations and even the established facts have incited little general interest.

LECTURE I

It is not my main objective in these two lectures to cover the vast amount of experimental work in the field of cancer, for this would be impossible. Rather shall I attempt to present some fundamental facts established by the experimental approach to the problem which have formed the basis of an hypothesis on which we have planned some of our recent investigations.

The experimental investigation of malignant disease is of fairly

[1] Delivered under the William Sydney Thayer and the Susan Read Thayer Lectureship in Clinical Medicine, March 8 and 9, 1934.

recent development, having had a small beginning in the early years of the present century following the discovery of the transplantability of cancer in animals. There was a period when many workers were attracted to the field, but when it became evident that the solution was not immediately forthcoming, interest waned. With each new advance made since, there has been a repetition of this, with between times a complete lack of general interest, outside a very small group. It is only within the last decade that a wholesome and sturdy expansion has taken place, based on the very great human importance of the problem, with the full recognition of the difficulties in the solution, and devoid of the impatient attitude which characterized the earlier period. Nevertheless, from the beginnings, behind the scenes, an earnest group has painstakingly laid a solid foundation of fact on which any future investigation must be built.

As indicated in our title, there have been four main lines of attack, carried on more or less independently of each other, which may be treated as four almost distinct chapters in a discussion of the subject.

Transplantable mouse tumors

The first chapter might be entitled the *Biology of the Malignant Cell.* The knowledge accumulated is based on the study of the transplantable mammalian cancers and naturally deals with the interrelationship between the tumor and the host. Jensen, Loeb, and Borrel were the first to give unquestionable proof that malignant tumors in animals could be successfully transplanted in series. While animal tumors had been known prior to this, an exhaustive reexamination of such growths was made following this discovery to determine whether or not they represented the same type of disease process as cancer in man. Occurring naturally, they were found to invade, metastasize, to arise in various tissues, and to have as large a range of variation in histological structure as the human varieties. There is no doubt now that the malignant tumors of the lower mammals represent the same process as the disease in man.

In the investigation of the transplantability of tumors, the first concern was the search for an infective agent. Numerous bacteria, several varieties of spirochetes and other organisms were described, but on final analysis all of these appear to have been eliminated. It

was definitely established that these tumors can be transmitted from animal to animal only by grafts of living tumor cells, and that the resulting new tumors are formed entirely by the multiplication of the inoculated cells. Exhaustive study has failed to produce any evidence that the cells of the host ever become cancerous by the intimate contact with the malignant tissue which it supports and by which it is being invaded.[2]

The laws governing the transplantability of these tumors are essentially the same as those limiting the transplantability of normal tissues. For example, using mixed stocks of mice, only an occasional spontaneous tumor proves to be transplantable and this occasional one takes only in a small percentage of the first group inoculated. It is now known that the primary factor involved is the genetic relationship between the new host and the individual originating the cancer cells. In pure line strains, where the animals have a uniform genetic make-up, any tumor occurring in the stock is transplantable to every other animal. By the study of the transplantability of tumors from two such pure line strains in various degrees of hybridization, where the parent stocks were resistant to each other's tumors, in some instances even the number of genetic factors involved in resistance has been worked out. Repeated transplantation over a long period may lead to some modification or adaptation to transplantation, but this does not extend to a point where the tumors may be successfully grafted to other even closely related species. The grafting of normal tissues is known to be limited in just this same fashion, but due to low growth energy, the transplantation cannot be carried far enough to test a possible adaptation.

With slight modification the histological structure of the original tumor is retained even after years of transplantation and passage through hundreds of animals. What may be called the biological

[2] The highly controversial interpretation of the rare instances where a sarcoma appears to develop in the stroma of a transplanted carcinoma may be an exception to this general statement. However, there is no agreement as to whether this sarcomatous change takes place in the stroma supplied by the new host or in that from the original host which was carried along with the graft. Other suggestions which have been made are: (1) that these tumors originally contained both elements but the sarcoma became dominant only on transplantation; (2) or that the apparent sarcomatous change is only a change in form of the cancer cells accompanying increased malignancy.

characters, such as general growth rate, range in variability of "takes" in mixed stocks, tendency to retrogress after periods of growth, etc. are so fixed and so characteristic for each tumor, that many can be differentiated from one another almost as easily as related strains of bacteria can be separated on the basis of their cultural characteristics.

From the point of view of the host's reaction to the transplanted tumor, three types of resistance have been recognized: the natural resistance, where no growth takes place, the acquired type, where after a period of growth the tumor is overcome and absorbed, and the experimentally induced immunity. In the light of later investigations it is probable that the so-called natural and acquired resistances are the result of the degrees of incompatibility between the genetic make-up of the host and of the tumor cells. The resistance induced by the previous injection of an amount of homologous living normal cells is not entirely understood. The study of resistance to transplanted tumors is of historical interest on account of the prolonged controversy between Bashford and the English school supporting what was called the "stroma theory," and Ehrlich, who had advanced the "athrepsia theory" as an extension of his famous side-chain theory. As neither of these attempted explanations has proved adequate, they will not be discussed here. Our own concern in this phase of the problem centered around the part played by the lymphoid cell in the phenomenon. Here it undoubtedly has a function in the mechanism by which the organism resists the growth of engrafted cancer cells, and according to Leo Loeb has an equally important rôle in resistance to normal tissue grafts. Of equal importance in this discussion is the fact that the induced resistance is as effective against grafts of normal tissues as it is for malignant cells (Rous).

The foregoing we consider to be the more fundamental points developed, but there have been many other important facts brought out from the study of transplantable tumors. Detailed histological study has failed to show striking specific characteristics which would differentiate the cancer cell from the actively growing normal one.[3]

[3] Dr. Warren Lewis has shown that the cells of certain connective tissue tumors grown *in vitro* exhibit certain differences from each other, and all differ in some particular from a strain of normal connective tissue. Whether these are greater than variation in connective tissue from different locations does not seem to be established.

While a different type of metabolism has been described for cancer cells, there is some doubt now as to the fundamental importance of this finding, for it has been demonstrated that certain very active normal tissue exhibits the same phenomena, and many malignant tumors failed to show these characters. The chemistry of the blood of animals bearing tumors, the chemical composition of the tumors including the various proteins, mineral salts, etc., have failed to give any fundamental differences from comparable normal tissue. Taking all of the data available from the study of the transplantable cancer one is impressed by the fact that, as far as we have gone, the malignant cell differs from the normal cell of the same tissue type principally in its possession of an excess of growth energy. It has been suggested that the latter property is due to its acquired ability to respire and metabolize under conditions not ideal for normal cells, but this property would seem to be a manifestation of its growth energy rather than a cause of it.

Induced tumors

The second phase of the investigation we shall discuss is the experimental production of malignant tumors. Cancers in man secondary to certain forms of chronic irritation or tissue disturbance caused by physical, chemical, or infective agents have been known for many years and are given special consideration in the textbooks on the subject. These include groups associated with certain occupations, as chimney-sweeps' cancer, mule-spinners' cancer, tar-workers' cancer, analine-workers' cancer, arsenic cancer, x-ray and radium cancer, etc. There is also a smaller group associated with irritation from certain parasitic infections, principally Bilharzia, syphilis, tuberculosis, and no doubt virus infections of the more chronic type may be followed by malignant changes on the same basis. In the earlier attempts to induce cancer experimental use was made of certain dyes known to have a stimulating effect on epithelial tissues, principally scarlet red, but here, in spite of very marked stimulation of growth, no instance of the process going over into a malignant condition was reported.

In comparatively recent years the results have been achieved by other means. The first demonstration of the experimentally induced

cancer was made by Fibiger, who showed that a malignant condition of the stomach of rats followed infection of the mucosa by a nematode isolated from cockroaches. A little later two Japanese workers, Yamagiwa and Itchikawa, succeeded in inducing cancer of the skin in rabbits by repeated and long-continued application of coal tar. These observations opened a new chapter in cancer investigation and a massive literature has accumulated on the subject. For our objective it is necessary to mention only a few of the more fundamental experiments.

There is no doubt that the tumors produced are true neoplasms, behaving essentially in the same manner as the spontaneous new-growths, in that they invade, metastasize, and eventually cause the death of the animal. Both carcinomas and sarcomas have been produced. It became evident early in the investigation that the mechanism involved is not a matter of simple irritation, for on fractionating the tar, the low boiling point portion, by far the most irritating, is inactive, or relatively so, in the production of tumors. On the other hand, the high boiling point fraction, which causes little evidence of gross irritation when applied to the skin, possesses considerable carcinogenic properties. I know of no better example of the value of persistent and systematic investigation than that which has led to the opening up of this important phase of the problem.

Under the leadership of the late Dr. Leitch and his successor, Dr. Kennaway, a group of workers in the Cancer Hospital Laboratories of London, by fractionating the complex mixture contained in coal tar, have succeeded in isolating the compounds responsible for the carcinogenic properties. By the use of the fluorescence spectroscopy it was found that many of the carcinogenic mixtures had a common spectrum. From this lead Cook was able finally to prepare a pure hydrocarbon which will induce malignant transformation with great regularity and in a shorter time than by the crude mixtures. It now appears that there is a group of related hydrocarbons present in coal tar with varying degrees of cancer-producing properties, having in common the phenanthrene ring system. Some of these compounds prepared synthetically possess equal carcinogenic properties. The significance of this observation is indicated by the fact that a number of naturally occurring compounds, some of them having considerable

biological activity, are known to contain this same characteristic ring system. Among these are the ovarian hormone, possibly the male hormone, and Vitamine D. As a suggestion of a further link between the tar product and the naturally occurring hormone, it is known that certain of the carcinogenic compounds may produce effects in the body similar to those produced by estrin.

The observations with the pure hydrocarbons have confirmed the suggestion derived from the earlier work that gross irritation as such plays no important part in the development of induced cancer, for these pure substances are without demonstrable irritating effect. Another important outcome of this study has been the failure to find any transitional step between the normal and malignant cell. There seems to be a direct mutation from one type to the other.

In summing up, the latter findings, in the study of the so-called carcinogenic substances, are of too recent a development to have received their evaluation in the general consideration of the mechanism of malignancy, but they open up a tempting field for speculation.

Genetic factors

The third important branch of cancer investigation is that dealing with the part played by inherited susceptibility. This important phase had its beginning in the observations of Tyzzer and of Murray, who found a significantly higher tumor rate in mice whose mothers and grandmothers had had cancer than in mice with no cancer in their immediate ancestry. The more significant study of the genetic factors involved, based on the study of strains, was probably the outgrowth of an unconscious experiment carried out on a small New England farm. Leo Loeb, attracted by the apparent endemic occurrence of tumors in this stock, undertook a more systematic study, while at about the same time Miss Slye started her extensive researches, the farm strain serving as one of her three parent stocks. For a number of years now the subject has been investigated by other workers trained in genetics, among whom may be mentioned Lynch, Marsh, Strong, Curtis, Dunning, and Bullock, and Little and his collaborators.

For the positive accomplishment in this line we have undoubted proof that it is possible to segregate strains having a very high spon-

taneous cancer incidence, and strains practically free from the disease. The question of infection or contagion has been eliminated by extensive and well controlled tests. The rate for the low strains is unaffected when they are kept in contact with the high strain, or suckled by cancerous mothers, or by a variety of other attempts to infect the low strain. The high strains in general are no more susceptible to inoculated or induced cancer than the low strains. The most striking fact which has developed is that the inherited tendency is not a general susceptibility, but is confined to an organ or a special tissue. Due to the external manifestations the first strains isolated were susceptible to breast cancer. Later strains with lung, liver, stomach, and several other tissue types have been developed by selective breeding.

Aside from the study of the nature of the inherited conditions, these stocks of mice of known tumor incidence offer splendid material for investigation. As an example, showing the part played by the physiological activity of a tissue, the mammary tumor strains have yielded important facts. Here the males under normal conditions do not develop cancer, but if castrated early in life and an ovary engrafted, a definite percentage of males do develop cancer of the breast. Females subjected to forced breeding have a higher rate than those bred at the normal rate. On the other hand, females of this strain, prevented from breeding, have a distinctly lower rate, and when castration is performed in early life, the rate is still lower. That these results are dependent on the amount and activity of the breast tissue, rather than to any general influence of ovarian secretion, is shown by the fact that such procedures as castration or intensive breeding have no evident effect on the cancer rates in organs or tissues other than the mammary gland.

When we come to the analysis of the data from the point of view of the genetic factors involved in the inherited susceptibility, difficulties have been encountered. As an example of the differences of opinion in this field, Slye, on the basis of a very large amount of material, claims to have established that inheritance occurs according to the Mendelian law and is a recessive character. There has been considerable criticism of Slye's basic data among geneticists, and her conclusions are not generally accepted. Lynch, with far less ex-

tensive but more carefully controlled material, including both mammary-gland and lung cancer, reached the tentative conclusion that susceptibility is probably semi-dominant in character and is unquestionably inherited, a conclusion which is shared by other workers.

Without attempting to enter into this difference of opinion it may be said that the ideal conditions for determining the exact mode of inheritance are not at present attainable. The difficulty lies not only in the lack of strains in which 100 per cent have the character and of other strains in which 100 per cent are without it; there is the additional disadvantage of dealing with a character which does not show itself until middle age, and may not appear until even very advanced age. An animal dying well past middle age of another cause, if it had lived even a few weeks longer, might have developed cancer. The results, referred to a few moments ago, in relation to the effect of breeding on the mammary tumor rate in strains having a normally high rate suggest that the manifestation of susceptibility requires something to set it off. This balance of tissue may be likened to a cocked pistol, with the more susceptible condition a hair trigger affair set off by a slight pull. In these cases the balance is so delicate that the strain incident to normal physiological activity is sufficient to cause the break.

This conception led to the belief, that, if some controllable method could be found of "pulling the trigger," or testing susceptibility, variable factors would be eliminated, and there would be a greater chance of determining the character of the inherited tendency. There have been two developments in this direction. At the Crocker Laboratory a study has been made of the sarcomas which develop in rats around tape-worm cysts of the liver. At the moment it appears that the differences in tumor incidence between strains is dependent only upon susceptibility to the parasite and the longevity of the animal. With massive infection of the liver, tumors are more apt to occur than where only a small number of cysts are present. If this interpretation is correct, a condition analogous to the tar-induced tumors would exist where normal resistance may be destroyed by long continued application.

The other investigation seems to offer a better method of determining the inherited susceptibility. Lynch had by selective breeding

isolated a family of mice which, under normal conditions, developed primary cancer of the lung in about 37 per cent, and another family with a low rate, something under 7 per cent. These tumors appeared in middle life, rarely if ever before one year of age. A method had been developed at the Rockefeller Institute for the induction of lung tumors, which consisted of a special system of applying tar to the skin. By employing this method with young mice of the two strains and killing them before normal tumor age was reached, the high strains were found to show primary lung cancer in almost 100 per cent and the low strain in 20 per cent of cases. Crosses and back-crosses between the hybrids and the parent strains have furnished test material, which show that susceptibility to induced tumors is inherited, and again the evidence points to a semi-dominant character. A further important observation has been made with this material. The high lung tumor strain has proved to be highly resistant to induced skin cancer, while a certain low strain has a marked susceptibility to this condition. This observation probably definitely eliminates the possibility that the inherited condition is dependent on a single gene. With this combination, even if the final work on the genetic factors is not forthcoming, we may at least expect some evaluation of the inter-relation of the two important factors in cancer causation, inherited tendency and tissue disturbance (irritation?) which have been considered more or less unrelated.

I hope that, in this very sketchy account of the development of three of the important branches of cancer work, sufficient material has been presented to indicate the basis for the almost universal reaction of the group of workers in experimental cancer research. This opinion, or even conviction, was that the possibility of a parasitic causal agent was eliminated from any serious consideration. The evidence, based on the investigation of the transplantable mammalian tumors, is that the tissues of the host, supporting and being invaded by the malignant cells, show no evidence of becoming malignant by this intimate contact. We have more direct evidence in the fact that, even with highly malignant tumors and very susceptible animals, thousands of attempts to transmit the disease have failed except under conditions that assure the introduction of intact, living cancer cells. We have seen how strictly transplantation is limited,

and how far the cancer cell is removed from the completely lawless individual it is supposed to be. There are examples where the experimental transmission of parasitic disease is more or less limited to species, but there is no example, where, given the conditions we have of cancer in mice, transmission by an infectious agent is not accomplished with ease. For a parasite to be involved in the experimentally induced tumors we would have to assume a ubiquitous organism, always present in all parts of the body, for, given proper conditions, tumors may be induced in any location. With the material collected from the genetic investigation the possibility of the infective nature of the disease was examined most carefully: young suckled by cancerous foster-mothers, cage contacts, and every possible method of intimate contact, without one jot of evidence in favor of an infective process.

Besides the evidence of organ specificity of the inherited tendency from the experimental material we have such natural examples as tumors in homologous twins in man. Among thirty-eight cases reported in the literature, in twelve only, one of the pair had cancer but, as the unaffected individual in all but one instance was still living at the time of the last report, the figure is not final. In the remaining twenty-six pairs tumors were present in both individuals; the growths in general were of the same type, in the same organ, and appeared at approximately the same time in each pair. This striking manifestation speaks strongly in favor of a genetic control and against exogenous agents in the form of parasites.

Can any real hypothesis be built up on the basis of the knowledge of the cancer process accumulated from these extensive experimental studies? I think the answer would be unanimous that no concrete theory could be formulated, but that the evidence indicates that the process is essentially a perverse physiological one, the understanding of which would have to await the more complete knowledge of the normal physiology of the cell. Without information as to why normal cells grow in an orderly fashion, and growth is checked when certain bounds are reached, how can we follow the lack of this property in the cancer cell? One observer has suggested that it may well be the lack or the loss of something in the cell rather than the presence of a specific stimulant to growth which accounts for the malignant process.

There are some indications in the presented data from which we might conclude more specifically that the malignant process is what is indicated by this suggestion—that it is a breakdown in the internal balance of the cell. On the basis of the genetic studies, the condition inherited, according to this idea, is a deficient balance in certain tissues. This conception is supported by the demonstration of the ease with which the susceptible cells in such individuals are changed to the malignant type.

The foregoing represents somewhat the state of cancer research and the opinions held (and still held by the majority) when the discovery was made which led to the next important phase of the experimental investigation of malignancy.

Fowl tumors

This, the fourth chapter, had its beginning with the transplantation of two avian tumors in 1910. A Japanese investigator, Fujinami, succeeded in transplanting a myxosarcoma, and later in the same year Rous of the Rockefeller Institute reported the transmission of a spindle-cell sarcoma. There was no indication that these accomplishments were of any greater importance than the addition of more transplantable tumors for study. The Rous tumor, which is designated as Chicken Tumor I, was the more closely studied in the early stages and, judged by the knowledge of mammalian tumors, represents a typical neoplasm. This was particularly true in its behavior on transplantation. The tumor occurred in a pedigreed stock and in the first series of inoculations it grew only in blood-related fowls, and failed in other strains of the same variety. There was a slow adaptation, so that after a time it would grow in the pure blood varieties and finally in chickens of other breeds. It was shown that the inoculated cells survived and multiplied, which led to the belief that the new tumors were formed from multiplication of the inoculated cells. The tumor invaded surrounding structures, metastasized more or less freely, and examples of such metastases arising from actual emboli of tumor cells were demonstrated. Comparing the chicken tumor at this stage with mammalian tumors, it was perhaps more malignant and metastasized more freely than any other, with the possible exception of a rabbit tumor.

The extreme malignancy of this growth led us to attempt again what had been so often tried in mammalian tumors, that is, the possible transmission by agents separable from the tumor cell. In the following year Rous succeeded in transmitting the tumor by filtrates, and I succeeded with desiccates of the tumor. Between these two methods the possibility of tumor cells being involved in the transmission seems to have been eliminated. The tumor transmitted by the agent has the essential characters and malignancy of the original newgrowth, and it was further established that, with serial transmission by the agent without cells, the agent multiplied or was increased.

This discovery brought out a division of opinion among investigators. One group accepted the chicken tumor as a true neoplasm, transmitted by an ultramicroscopic organism or virus, and felt that all of the evidence against the parasitic cause of cancer in mammals must be re-examined. The other group considered this tumor an infectious granuloma, having no relation to true malignant tumors, and that it rightly belonged to the virus group of disease. I admit that, if we had not gone beyond this stage and if there had been no other types of tumor transmitted by this means, I would have been in the latter group. However, within the next three years three distinct types of chicken tumors were transplanted at the Rockefeller Institute, one by Tytler, one by Lange, and a third one by Rous and Lange. Rous and I, by 1914, could report three distinct types which, with all of their histological and biological characters, could be transmitted equally well by the separated agents as by grafts of tumor cells.

Two striking examples may be mentioned. Our Tumor No. VII was designated as an osteochondrosarcoma, and showed the full range of differentiation between cartilage, bone, and bone marrow. The cell-free extract of this tumor, injected into muscle, remote from any pre-existing bone or cartilaginous structures, induced the growth and differentiation of the local cells into bone, cartilage, and bone marrow. Another tumor which arose in smooth muscle, with a characteristic histology, and which on transplantation showed a tendency to metastasize to muscles at the tendon junctions, had all these characteristics transmitted by its extracts. There is now a

formidable group of these fowl tumors, yielding in each case an agent capable of transmitting all of the essential individual characters.

What is to be said as to the nature of this group of agents, and what relation have these tumors to mammalian neoplasms? In regard to the latter question, through the demonstration of the transmission of various kinds of histologically typical tumors, and the similarity in properties between these and mammalian tumors on transplantation, there has come to be an almost general acceptance of this group as true neoplasms. The failure to transmit mammalian tumors by agents independent of the cell, as negative evidence, does not seem to be at present sufficient ground on which to exclude the fowl tumors from the group.

As to the nature of the agents, even a tentative answer is not so easy. Until recently they have been accepted almost without question as filterable viruses, and they have in common with the typical representatives of this group their ultramicroscopic size, filterability, and their dependence on living cells for their multiplication. If Chicken Tumor I were the only type of tumor transmissible by a separable agent, it would not be difficult to accept the idea of a living virus as the causal agent. The difficulty comes when one attempts to expand this conception to cover the varied types of tumors, each with an agent, not only limited in its action to a species and to definite cell groups in the organism, but with the property of causing them to multiply and differentiate into various complex tissue types. For the chicken tumor group alone we would have to consider a number of varieties of such viruses, and if the idea is accepted for other species, to explain fixed individualities, histological and biological, we would have to imagine almost a separate variety of virus for each tumor. In fact the chicken tumor group may be considered as giving evidence against the parasite theory. An already differentiated cell becoming infected with a growth-stimulating parasite might be expected to retain its essential characters in the malignant state, but with the fowl tumors we have evidence that the complex differentiation of the cells is just as much the result of the agent as is the growth. This would indicate that the agent is probably closely related chemically to those factors normally determining cell differentiation. It was on the basis of these properties of the agent

that in the early stages of the investigation I found it difficult to accept the virus theory.

Gye, in an attempt to eliminate the objection based on the multiplicity of types, devised a complex theory, with a single virus on one side capable of forming a union with some factor of the cell, which gave it specificity. The evidence for the existence of the multiple factors faded under close examination. Recent findings, which include McIntosh's demonstration of filterable agents in tar-induced fowl tumors, have reduced Gye's conception to a point where we agree with Professor Boycott when he remarked that "when it became necessary to postulate a normal virus occurring in normal cells, one had better call it something else than a virus."

You can readily see the difficulty at this stage in formulating any conception of the factors involved in malignancy. My opinion was that further progress depended on the formulation of some working hypothesis, and this seemed impossible unless the chicken tumor could be brought into line with the mammalian tumors or definitely eliminated as representing a different type of disease process.

To-morrow I shall review our attempts to bring some unity into the problem and some of the developments that have been the outgrowth of these attempts.

LECTURE II

Yesterday I attempted to give you some idea of the facts brought out by the experimental investigation of the problem of malignancy, which serve as a basis for certain convictions, the tests of which will be discussed in to-day's lecture. We have a mass of information accumulated from the three important lines of investigation based on mammalian cancer, which seemed to eliminate the possibility that a parasite or exogenous factor played any rôle in the essential mechanism involved in malignancy. On the other hand, we have the positive evidence which seems to point definitely to the interpretation that the process is a perverse physiological phenomenon, involving probably the internal control mechanism of the cell. Such a conclusion would lead one to believe that further advance in the definite solution of the problem would depend on the understanding of the normal physiology of the cell. In conflict with these conclusions were the

findings with the avian tumors, where it was shown that transmission could be effected through filterable agents separable from the tumor cells. Our reason for doubting the almost generally accepted idea that these agents were viruses was discussed.

If we tacitly accept the chicken tumors as true neoplasms and reject the idea that the filterable agents are viruses, what possible alternative hypothesis can be developed as a working basis on which to plan further investigations? Let us consider the possible mechanism in its simplest form, by which a normal cell may be transformed into a malignant one on the basis of the conception of a perverse physiological process. I think I am correct in saying that most, if not all, well understood physiological functions are balanced phenomena. Perhaps the simplest example is the rhythmical contraction of the heart, the rate of which is controlled by two forces, one which tends to accelerate and the other to retard. If we assume that the control mechanism of the cell is composed of two factors, there would be two possible ways in which the balance could be interfered with —either the loss of the retarding factor or the increase of the accelerating factor. This simple hypothesis has been subjected to experimental tests.

If this conception is correct, the factors controlling the cell might be expected to have properties in general similar to other physiologically active cell products, such as hormones, enzymes, etc. It was on this basis that we undertook what appeared to be an unprofitable task of devising tests which would give comparisons between the chicken tumor agents, on the one hand with the typical animal viruses, and on the other with the physiologically active cell products. The results of these experiments, based on perhaps unwarranted assumptions, will be presented for your consideration.

The first test was carried out on the basis that many of the active biological agents of cell origin, which are more or less selective in their action, are known to be adsorbed, fixed, or inactivated *in vitro* by specific substances on which they normally act. There is not this affinity or interaction with other materials. Experiments devised to test the tumor agents from this point of view, carried out with Duran-Reynals, gave the following definite results. The sarcoma agents were found to be adsorbed, fixed and inactivated *in vitro* by mesodermal tissue

of susceptible fowls, but not by similar tissues from nonsusceptible birds or other animals. Furthermore, the epithelial tissues or organs from susceptible chickens were without effect on the tumor agents. In addition to this observation Deelman has noted the fixation of the tumor agents by chick embryo tissue, and Pentimalli, by embryonic and adult connective tissues. Control tests with typical viruses and tissues most receptive to infection showed that the viruses were not inactivated, but by this contact had their infectivity greatly increased.[4]

From the point of view of the test, the tumor agent seems to behave like many of the cell products and differs essentially from the viruses in this particular.

The second group of tests were carried out with Dr. Gates and Mr. Sturm and had as its basis variations in the activity of different wave-lengths of ultraviolet light on bacteria and other infective agents. By plotting the energies required to kill or inactivate against the corresponding wave-lengths, similar graphs are obtained for comparing the qualitative and quantitative action of ultraviolet light. Gates had previously shown a curve similar in both respects for a variety of organisms, of viruses and of phage, which suggests the presence of a common factor or closely related chemical entities present in these forms, sensitive to ultraviolet light. The accumulated data for the tumor agents give a curve showing that inactivation requires a vastly greater amount of energy, and furthermore that the form of the curve is fundamentally different. The latter suggests that inactivation is due to the destruction of a substance having an essentially different spectral absorption and therefore being of a different chemical character. A preliminary study, devised to compare the deduced ultraviolet spectral analysis from the foregoing data with the actual coefficients of absorption of the highly purified tumor agent, yielded most suggestive results. There were not only absorption bands corresponding to the most active wave-lengths, but these were ob-

[4] This later result has opened up a most important investigation in demonstrating the existence in certain tissues, particularly in the testicle, of a factor which has become known as the Reynals factor, having as its chief character the property of increasing tissue permeability and of enhancing the infectivity of viruses and bacteria.

served to fade on standing, probably paralleling the loss in tumor-producing activity of the solution. Unfortunately this work was halted by the untimely death of Dr. Gates.

In these tests, as with the first, there seems to be a sharp division between the parasites and the tumor agent. Although there are not very accurate data, the indications are that ferments and antibodies require even more ultraviolet light energy for their destruction than the tumor agents. The result as it stands, while it has received less notice than some of the other studies, seems to me the most important in suggesting a fundamental difference between the tumor agents and the typical virus group.

Encouraged by the two foregoing studies, we next considered the possibility of isolating the active principle from the crude tumor extracts. As you know, extensive investigations are under way directed towards the clarification of the nature of enzymes, hormones, vitamines, and a variety of other physiologically active products. The indications are that some of these are either of a protein nature or are carried by proteins. With this possibility in view for the tumor agents, we attempted a fractionation of the tumor proteins. It proved comparatively easy to precipitate out a fraction which carried all the activity of the extract. The fraction, however, proved to be complex. It was either a mixture of muco- and nucleo-protein, or was a protein of unusual composition. The fraction could be somewhat purified by being repeatedly dissolved in alkali and reprecipitated, but the means necessary to separate the fraction further were too drastic for the survival of the active principle. Further study of this fraction was rendered unnecessary by the next step.

In the hope of eliminating some of the inactive material from the tumor extract we made use of the well-known method of adsorption on aluminum hydroxide. Other investigators had attempted the use of this method. So little of the active material was releasable from the aluminum that only an occasional tumor could be induced. From this point of view the method failed to help. However, we took the precaution of testing the activity of the supernatant fluid after the removal of the aluminum and its adsorbed material. This proved to have a tumor-producing property even more pronounced than the original extract. This fraction, which we shall call the aluminum

supernatant, represents a substantial step in the purification of the agent. On examination, it was found that the aluminum had removed practically all of the proteins. Not only were all the chemical tests for these substances negative in the aluminum supernatant, but guinea-pigs injected with as much as 40 cc. of this fraction failed to show any sensitization to a second injection, and other tests, in which repeated injections into rabbits were made, failed to show any evidence of the development of complement fixing antibodies. If we may accept the figures given in the literature, this would indicate that the 40 cc. of the aluminum supernatant contained less than 1/20,000,000 of a gram of protein, and the failure of the complement fixation test indicated a concentration of less than one part in 1,000,000,000.

As to the character of our aluminum supernatant, it was a clear, colorless fluid, retaining the viscous property of the original extract. It was established that the viscosity was due to a chondroitin-sulfuric-acid-like compound. The attempts to remove this substance by the usual chemical methods proved too rigorous for the agent. However, Claude conceived the idea of combining this acid substance with a basic protein, with the hope that the new compound might be precipitated at a pH tolerated by the agent. This proved to be the case when gelatin was added. The viscous material was completely removed, leaving a clear, limpid material with its tumor-producing activity unreduced. Thus another ingredient was eliminated. Without going into details, it has been shown likewise that fats and lipoids apparently have no part in the activity.

The last step completed was reached by starting with our aluminum supernatant fluid, which was rapidly dialyzed in collodion bags against running distilled water (Northrop and Kunitz method). By this means, with no reduction in the concentration of the active principle, 70 per cent of the solids left after adsorption were removed. The total solids per cc. in this active fluid are 0.44 mg. with a nitrogen content of 7.4 per cent. By elimination of the chondroitin-sulfuric acid and lipoids known to be present, we may reduce the amount of material concerned with the active principle by another 25 per cent. Thus the total activity of an extract may be carried by a fraction which represents 0.2 mg. per cc., and 0.2 cc. of such an extract will

produce as large and as active a tumor as the same amount of the original extract. While it is highly improbable that we shall arrive at a very definite idea of the chemical constitution by the methods we are using, yet some idea of the type of substance may be reached.

The value of this attempt, aside from its evident aim, is again the contrast between the tumor agents and the typical viruses. While it has been claimed that protein-free viruses have been prepared, it must be remembered that these agents are infective in extremely high dilutions, and the evidence of this dilution is shown in each step of the so-called purification in the more carefully checked studies with typical representatives of this group. I wish to emphasize again that the tumor agents have been brought to the present stage of purity with no detectable loss in activity whatever, and reduction in activity by dilution of this agent is easily detected.

Our extensive study of the antigenic properties of the tumor agent will not be taken up. Certain English investigators are laying great stress on this approach to the nature of the tumor agents. But they have continued to use crude extracts and to draw conclusions in spite of our evidence that even the partially purified agent is without antigenic properties. Even without this, the results are too complex to be of any value at the moment. It seems unlikely that this approach will add information as to the nature of the agent, particularly as it now seems established that antibodies may be developed against enzymes and probably against hormones as well.

Before taking up the next property of the tumor agent to be discussed, may I return to our very schematic hypothesis? When this possible interpretation of the relation of the tumor agent to the normal control mechanism of the cell was first presented in 1928 at the London Conference, the suggestion was made that the best support to such a hypothesis would be the demonstration of the other part of the mechanism—the retarding or balancing factor.[5] There was no evidence of such a growth-retarding substance at the time, and it was only later by accident that indications were found of the submerged inhibiting factor associated with the tumor agents. It came about in the following way. Not infrequently in the transmission of the fowl

[5] An editorial in the *London Times*.

tumors we encounter a desiccate which yields an inactive extract, or one of very low potency, for no apparent reason. It was first noted that the residues left after the extraction possessed active tumor-producing properties. This was puzzling, as the extracts contained about 60 per cent of the soluble material, and there seemed no reason why in one case the agent was carried with the soluble fraction and in another it was not. To throw some light on this question, attempts were made to get the agent out by making a second extraction of the residue left after the first extraction. This proved successful and the residue extracted a third time proved even more active. On the basis of this it might be supposed that the agent was soluble only with difficulty, but on examining the results from the inoculation of the residues left after each extraction this explanation proved inadequate. This finding was that the tumor-producing activity of the residue increased after each extraction. There seemed no other deduction from this than that an injurious or inhibiting factor, more soluble than the agent, was present in some tumor desiccates, and on account of its solubility came out in sufficient amounts in the first extract to neutralize the agent. Its removal left the agent in the residue untrammelled and therefore more active. This interpretation was confirmed by the following investigation.

In reporting the attempts to purify the active principle, I told you a few moments ago that, when an active tumor extract was treated with aluminum hydroxide, in spite of the fact that some of the agent was carried along with the adsorbed material, yet the remaining extract had a greater activity than the original solution. We have had a number of examples of an inactive extract which, after treatment with aluminum hydroxide, showed an activity comparable to the most potent materials. In fact, almost any extract or filtrate has its potency increased by this treatment. That this result is due to the removal of an interfering factor is demonstrated by the fact that the adsorbed material, released from the aluminum, in proper concentration is capable of neutralizing the most active tumor extracts. From the analysis of a large amount of data, it would appear that the activity of any given extract or filtrate depends on the relative amounts of the two factors present. It has proved easy to test the amount of inhibiting factor in any given sample. While the agent is

destroyed by heating to 52°C., the inhibitor will withstand 60° or more. Therefore, with the tumor-producing agent destroyed by heat, the inhibiting action of the extract on a standard active product will give indications of the relative amount of inhibitor present with a fair degree of accuracy.

Examination from many other angles, checked by a large number of tests, leaves little doubt of the existence of an inhibiting factor associated with the chicken tumor agent. Similar conclusions have been arrived at by Gye and Purdy, and also by Sittenfield, Johnson, and Jobling.

At this stage where did we stand in regard to the hypothesis on which the investigation had been based? For the first objective, while the properties of the tumor agents in each condition tested differed essentially from those of typical viruses, it is probable that no one of these differences alone definitely separates the two groups, yet taken together they represent, we think, more than just indications of an essential difference between the two. Formulated in a definite statement, the acceptance of the suggested interpretation means the assumption that the product of a characteristic cell (malignant) acts on an undifferentiated cell, causing it to assume the peculiar characters of the specialized one, and in this new form the cell produces more of the agent capable of inducing the transformation of other cells. Furthermore, the new cell, under the conditions of this hypothesis, must pass all of the properties to its descendants. The acceptance of this hypothesis means the acceptance of the possibility that an inanimate substance for all intents and purposes reproduces itself. At the time we first brought forward this suggestion, there were no accepted examples of such a phenomenon, and I had some sympathy with one critic who said that it represented "too great an innovation in the order to things to be accepted." However, a little later a well authenticated example of just such a condition was established in the field of bacteriology. This is too well known now to require more than a passing comment. In the pneumococcus group of bacteria, there is the avirulent, non-capsulated form, and several serologically distinct, pathogenic, capsulated forms. It has proved possible to extract an agent from the specific forms which, when brought in contact with the non-specific form, causes these bacteria to assume all of the

characters of the special form, including the production of more of the agent, and the power to pass on these properties and abilities to its descendents. There are other well established instances in the field of bacteriology of similar phenomena which are clear examples of presumably inanimate agents which appear to multiply, but which may be said to be multiplied or to stimulate their own reproduction. It seems highly improbable that these specialized agents can be shown to be viruses in the accepted meaning of the term, yet their ability to stimulate their own production is well established. We have suggested the term, *transmissible mutagens*, to describe this group of agents, and are of the opinion that the tumor agents may belong here rather than in the virus group as it is at present constituted.

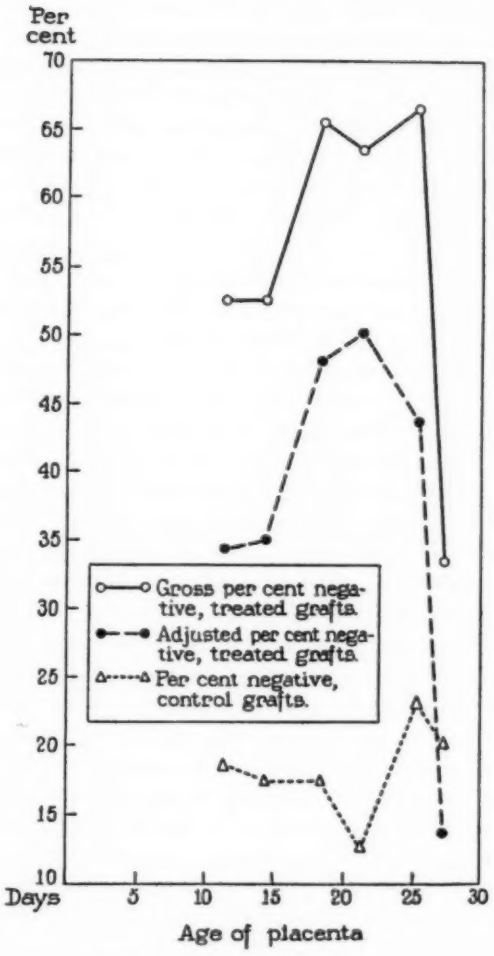
Leaving this hypothetical discussion, let us continue the investigation. It was next established that the inhibiting factor from one type of fowl sarcoma was equally effective in neutralizing the agent of another type, in spite of the high degree of specificity shown by the agents themselves. This investigation was extended to test the limitation in the action of the inhibitor on various types of mammalian tumors. These products of chicken sarcomas proved to be equally effective in preventing or limiting growth of certain mammalian sarcomas, but were found to be without effect on carcinomas. While the variety of tumors tested is not large, yet it may be tentatively accepted that, as indicated, these inhibitors are tissue limited but not species limited. These results represent perhaps the first definite connecting link between the mammalian and fowl neoplasms. Added to this evidence we have recently found some evidence that a similar agent from a rat tumor is effective in inhibiting the growth of a mouse tumor.

The next question, is there any evidence that the two factors demonstrated in the fowl tumor have any relation to the assumed two factors which control the growth and differentiation of normal tissues? If from the latter tissue the two factors could be extracted, separated, and concentrated, the following conditions might be expected. The growth-accelerating portion, brought into relation with the right cell type under ideal conditions, should cause a break in the balance, with active growth resulting. The retarding factor should be effective in neutralizing the growth factor in malignant cells. With the first

proposition, working entirely in the dark as to proper methods, we have had an occasional development of malignant tumors resulting from the injection of a fraction prepared from an active normal tissue of the testicle of the fowl. While something over twenty tumors have been induced by this material, in four positive experiments, yet this represents only about seven per cent of the attempts to accomplish this purpose. We now consider that these results have not been reproduceable with sufficient regularity to be given serious consideration in this discussion. On the other hand, there is considerable evidence of an inhibiting factor from normal tissues which represents the last completed phase of the investigation.

On the assumption that the greatest concentration of the balancing factor would be present in tissues showing the strongest growth energy, our search for evidence of inhibitors in normal tissue was made with those showing active growth. The results have been too clear-cut to require extensive presentation of the data. Without going into the various tissues tested and the methods employed, the positive findings were as follows. An inhibiting factor or substance has been found in placenta and embryo skin which exerts a definite action on transplantable mammalian cancers, but is without effect on mammalian or fowl sarcoma. As with the inhibitors from fowl sarcoma, the effectiveness is tissue limited but not species limited. For example, the products from mouse, rat, or rabbit seem equally effective on mouse tumors. As the placenta offered not only the most potent but the most easily collected material, our later experiments were confined largely to this material.

At this stage it was necessary to obtain larger amounts of material than the small laboratory animals provide. Tests were made with human placenta at term, and the early cow, sheep, and hog placenta available in slaughter-houses. From none of these did we secure effective amounts of the inhibiting factor for mouse tumors, our standard test material. Was this due to the great gap between the species or was it a question of the age of the placenta? To answer this we have made a systematic investigation with rabbits. The placenta from this animal, with a thirty-day term, showed the first definite evidence of the inhibitor between the tenth and twelfth days of pregnancy. By the nineteenth day this had reached its maximum



TEXT-FIG 1. THE EFFECT OF AGE OF PLACENTA ON THE AMOUNT OF INHIBITOR FACTOR PRESENT

These data are based on rabbit placenta and a transplantable mouse carcinoma.

level which was maintained until the twenty-sixth day. Between the twenty-sixth and twenty-eighth days there was an almost complete disappearance of the factor (Text-fig. 1). We have no suggestion as to the significance of this finding, but it indicates that our failure with human and other animal placentas was probably due to the age rather than the species difference. This point, tested with cow placenta of an age corresponding to the active period of the rabbit, indicates the correctness of this conclusion.

We now come to the crucial test of the inhibiting factor from normal tissues. All of the experiments so far reported have been based on the "taking" of grafts of transplantable tumors and their subsequent rates of growth. The cells making up these growths are foreign to the animal supporting them and the factors involved in the necessary adjustment to growth are delicate ones. Fortunately, for a more crucial test we have at hand large numbers of mice with spontaneous or natural cancers, the by-products of the genetics investigations. There is no doubt that, as a disease mechanism, they represent the same condition as cancer in man. The one important difference to be contended with is the accentuation of susceptibility brought out by selective breeding, which gives us perhaps a more difficult problem than we would encounter if this were not the case.

The majority of our series are primary mammary tumors, and particularly with this form the natural history, the results of operative removal and various other points, have been extensively investigated. For a base line from which we may measure the results the following facts are well established. The general course of events, based on thousands of observations, is that a tumor of this class, once established, continues to grow until it causes the death of the animal. Spontaneous recovery is extremely rare; temporary retrogression, or even temporary cessation of growth are of unusual occurrence. Radical surgical removal is followed by local recurrence in about fifty per cent of the cases, and autografts re-inoculated following such an operative removal, which may be likened to an induced metastasis, grow in practically one hundred per cent. The greatest difference from the human disease, partly due to the accentuated susceptibility to malignant transformation, is that the tumors are frequently multiple, and there is a tendency to develop new areas of malignancy. The figures on this point will be given later.

On the basis of the established characters indicated, the action of the inhibitor has been tested on the following points: (1) Local recurrences following radical surgical removal (Table I); (2) The growth of autografts, controlled by the introduction of a second untreated autograft in each animal (Tables II and III); (3) The effect on the growth rate, when the extract is injected at a distance, with no local interference with the tumor (Table IV); and finally, (4) The effect on the rate of new tumors developed (Table V).

TABLE I

The effect of placenta and embryo skin extracts on local recurrence following surgical removal of spontaneous tumors of mice

	NUMBER OPERATED	NUMBER RECURRED	PERCENTAGE RECURRENCE
Controls:			
Murray	48	23	47.9
Haaland	174	96	54.0
Murphy and Morton	39	18	46.2
Nakahara	50	26	52.0
Present series	144	69	47.9
Total controls	455	232	50.9
Placenta treated	100	6	6.0
Embryo skin treated	59	15	25.9

For comparison with the present experiments data in the literature on the results of operative removal of spontaneous tumors without other treatment are included in the above table.

Murray, J. A., Third Scientific Report of the Imperial Cancer Fund, London, 1908, 69.
Haaland, M., Fourth Scientific Report of the Imperial Cancer Fund, London, 1911, 1.
Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, **22**, 800.
Nakahara, W., *J. Exp. Med.*, 1925, **41**, 347.

The results as presented in the tables seem so definite and clear-cut that further comment is unnecessary.

These very definite results with the spontaneous cancers, based on sufficiently large numbers to give significant figures, taken with the evidence from the transplantable varieties, leaves no doubt that certain normal tissues yield a substance or substances capable of retarding or preventing the growth of cancer cells in animals, and there is even some evidence of the prevention in the origin of tumors in highly susceptible stocks. May I ask that you do not consider these

findings from the point of view of their possible practical significance, but rather from their bearing on the theoretical discussion? The

TABLE II

Effect of embryo skin and placenta extracts on autografts of spontaneous tumors

	NUMBER WHICH GREW	PER CENT WHICH GREW	PER CENT DOUBTFUL OR NO GROWTH
46 tumors removed:			
Autografts treated with embryo skin extract	29	63.1	36.9
Autografts not treated	41	89.1	10.9
65 tumors removed:			
Autografts treated with placenta extract	21	32.3	67.7
Autografts not treated	53	81.5	18.5
64 tumors removed:			
Autografts, no treatment to graft or wound	62	96.9	3.1

	NUMBER	PER CENT
Autografts treated with embryo skin, negative or definitely smaller than control	33	71.7
Autografts treated with placenta, negative or definitely smaller than control	63	96.9

TABLE III

Growth of autografts of spontaneous tumors after treatment with placenta or embryo skin extract

	NUMBER OF AUTOGRAFTS	NUMBER WHICH GREW	PER CENT WHICH GREW
Controls:			
Haaland	62	60	96.9
Murphy and Morton	29	28	96.6
Nakahara	25	24	96.0
Additional Rockefeller Institute Series	202	192	95.0
Total controls	318	304	95.6
Placenta treated	65	21	32.3
Embryo skin treated	46	28	60.9

The fate of untreated autografts of spontaneous mouse cancer as reported in the literature is given for comparison with the fate of treated grafts.

chances are that a practical outcome is remote, yet if the principle suggested could be established, certainly an opening leading towards the solution of the problem might be expected.

Let us consider the inhibitors from the point of view of the hypothesis. In the first place, there is no conclusive evidence that the

TABLE IV

Effect of embryo skin and placenta extracts injected intraperitoneally on spontaneous tumors without local interference

	NUMBER OF TUMORS	CONTINUED GROWTH	STATIONARY OR SLIGHT RETROGRESSION	MARKED RETROGRESSION	COMPLETE ABSORPTION	TOTAL INHIBITED
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Treated with skin extract	67	32.8	28.4	14.9	23.9	67.2
Treated with placenta extract	75	30.7	21.3	26.7	21.3	69.3

Haaland found only 1.4 per cent of retrogressions in untreated spontaneous tumors of mice, based on 353 cases, while Woglom, with 2000 cases, noted only 0.8 per cent which failed to show a progressive course. These figures will serve as a base line for judging the results shown in the above table.

TABLE V

Development of new tumors in mice

	NUMBER OF TUMOR ANIMALS	NUMBER WHICH DEVELOPED NEW TUMORS	PERCENTAGE WHICH DEVELOPED NEW TUMORS
Controls:			
Haaland	209	73	35.0
Nakahara	50	16	32.0
Early Rockefeller Institute Series	138	53	38.5
Present series	125	54	43.2
Total controls	522	196	37.5
Tumors removed:			
One treatment skin extract	46	3	6.3
One treatment placenta extract	65	3	4.6
Tumors not removed:			
Several injections skin extract	42	1	2.4
Several injections placenta extract	57	0	0.0
Total treated	210	7	3.3

The development of new areas of malignancy, not metastases, in mice having one or more tumors at the time observation starts is not uncommon. This "new tumor" rate is contrasted with that of the treated animals.

normal cell is controlled by the interaction of the two assumed factors, yet there is strong indication that normally developing tissues are

controlled in their differentiation by extractable factors, as demonstrated by Spemann.[6] While we have inhibiting substances from tumors and from normal cells which act on malignant tissue, there is no evidence that they represent similar substances or that even their mechanism of action is the same. Therefore, any conclusion in regard to the suggested interpretation must be drawn with extreme caution. I have been asked what relation our previous work on the resistance to malignant growth has to the present study. According to our present ideas, our first study had to do with the body's reaction and attempt to control the local overgrowth. The present study has to do, we think, with the internal mechanism of the cell. Whether the first factor is operative through the agency of the second, we are not in a position to say.

From the more general consideration of the material presented to you, can any conclusion be reached? The evidence collected from the three main lines of investigation, based on mammalian tumors, was reviewed, and I hope conveyed to you the same feeling that it has to the majority of workers in the field, namely, that the idea of an exogenous agent or parasite was untenable, there being absolutely no evidence for and very strong evidence against such an outside agency being involved in the essential malignancy mechanism. If the fowl tumors be accepted as true neoplasms, the suggested classification of the transmitting agents as viruses introduced a different situation. The facts which led us to oppose the virus idea and the evidence indicating a different interpretation have been discussed. While we cannot claim that the different interpretation has been established, yet the sum of the collected data is sufficient to do more than simply indicate an essential difference between the tumor agents and the viruses.

In an attempt to bring the chicken tumor into relation with mammalian tumors, the failure so far to transmit the latter by agents separable from the cells must not be taken into consideration. My

[6] Spemann's "organizers" isolated from cells in certain areas of the early embryo cause growth and specific differentiation of cells in the embryos. In this respect the normal tissues yield an agent similar to the tumor agent, but the resulting masses from the "organizers" are more in the nature of benign tumors, and no serial transmission has yet been effected.

only comment is that the fowl tumor agent is quite delicate; in solution it rapidly becomes inactivated, and it will withstand very limited manipulation. It might be conceived that a slightly greater susceptibility to injury on the part of a similar agent for mammalian tumors would render it difficult to isolate and demonstrate by the present methods.[7]

It must be evident to you how enormous the gaps are in the supports for the hypothesis that the malignant process is a break in the internal growth control mechanism of the cell. It would be tempting to conclude that malignancy is due to the lack or inadequacy of a normal factor, rather than to the presence of a specific stimulator to growth, for this would offer encouragement and might give direction in the search for curative measures. In spite of some support for such a conclusion, based on the inhibitor factor, the direct evidence is too inadequate and the materials utilized are too complex to justify the acceptance of more than a tentative hypothesis on which to base further studies. The use of the hypothesis in this way has opened up a line of investigation which has added a few new facts which may prove to be important in the final analysis. That is all that can be claimed for it. As such it has proved of value, but its establishment or elimination must depend on further study.

[7] It is too early to judge whether or not the study of the wart-like disease of wild rabbits reported by Shope will throw light on this complex condition. This disease in the wild varieties behaves like a chronic infectious process, but under certain conditions such as internal autografts, assumes more neoplastic-like characteristics (Rous). It is not yet clear what relation this condition bears to true malignant tumors, or whether the infective agent is primarily concerned, or whether this represents a malignant condition developing in a chronic infectious lesion (Fibiger's nematode cancer of the stomach).

A THIRTY-MINUTE SPECTROGRAPHIC MICRO-METHOD FOR THE DETECTION OF PATHOLOGICAL LEAD IN PERIPHERAL BLOOD[1]

HAROLD BLUMBERG, Sc.D.,[2] AND T. F. McNAIR SCOTT, M.R.C.S., M.R.C.P.

From the Harriet Lane Home of the Johns Hopkins Hospital, and the Department of Pediatrics of the Johns Hopkins University School of Medicine

Submitted for publication November 8, 1934

In a previous report it was demonstrated that the spectrographic detection of appreciable amounts of lead in the blood constituted an aid in the diagnosis of plumbism (1). During the course of further investigations on the subject, the method was standardized for the quantitative estimation of blood lead (2). The analysis required the ash from 5 cc. of whole blood, and could be completed in twelve to twenty-four hours.

In this paper we wish to report a much more rapid method for the detection of pathological lead in the blood. The spectrographic work of Sheldon and Ramage (3), who were able to detect a number of elements in 0.1 cc. of normal blood, suggested that the same convenient amount might suffice for the detection of pathological blood lead during lead intoxication. This quantity of blood proved satisfactory, and the following method was evolved.

METHOD

The Instrument. The instrument used was a Hilger E1 large quartz spectrograph, operated in conjunction with a graphite arc and a quartz sphero-cylindrical concentrating lens.

The Electrodes. The electrodes employed were high purity graphite rods, 10 mm. in diameter. The upper electrode, the negative, was used with a flat surface. The lower electrode, the positive, was

[1] Aided by a grant from the Hartley Corporation.

[2] In conjunction with the Biochemical Laboratory of the Johns Hopkins University School of Hygiene and Public Health.

hollowed at its upper end by means of a steel drill, leaving an evenly cored space approximately 6.5 mm. in diameter and 3.0 mm. in depth. Before use the electrodes were cleaned by burning the arc between them for two to three minutes at a red-white heat. When cool, the unburned bottom ends of the lower electrodes were fitted snugly into bored rubber stoppers and inverted in acid-cleaned test tubes. In this way a number of positive rods could be kept free from dust and ready for use. After an analysis, $\frac{1}{4}$ to $\frac{1}{2}$ inch of graphite was clipped from the used ends of the electrodes before they were prepared for another analysis.

The Blood Samples. For each analysis three samples of finger-tip blood were obtained, preferably from different fingers. The tip of the finger was washed with 70 per cent alcohol and dried with a sterile gauze. The samples were collected in 0.1 cc. micro blood-sugar pipettes which had previously been cleaned in strong nitric acid, washed in distilled water and dried in hot air. The blood from each finger was delivered immediately into the hollow core of a prepared electrode, which was then replaced in the inverted test-tube and taken for analysis. By means of careful drainage, the pipettes were emptied so well that the amount of blood left adhering to their walls was of no practical significance.

The Analysis. With the blood in the core of the lower positive electrode, and with the flat upper electrode as the negative pole, the arc was struck. For about fifteen seconds the electrodes were scarcely separated, thus permitting the clotted blood to smolder and partly to char, without actually burning. The electrodes were then drawn apart and the arc allowed to burn. By visual observation of the light beam and of the burning electrodes, it was easy to tell when the sample had completely burned. This usually required thirty to forty-five seconds.

After the photographic plate was developed and fixed, it was rinsed in water and examined wet. If the blood contained a pathological amount of lead, the sensitive lead line at 2833.07 Angstrom Units would appear visible to the eye, and the minor lines at 2802.09 A.U. and 2614.20 A.U. could be seen with the aid of a lens. This was considered a positive result. In the control samples, on the other hand, the sensitive line at 2833.07 A.U. either did not appear, or showed as

a faint line visible only with the aid of a lens. The minor lines could not be detected at all. This was considered a negative result.

The total time required for the procedure was the sum of the requirements of the three steps involved. The three samples of blood could be taken in five minutes, the blood could be burned in the arc in ten minutes, and the photographic plate could be developed, fixed, and examined in fifteen minutes. The entire analysis, therefore, could be completed in thirty minutes.

NOTES AND PRECAUTIONS

The use of unashed blood necessitates the burning of organic matter and of a considerable amount of electrode graphite during the course of the analysis. When the graphite arc burns in air, it forms small amounts of cyanogen, the intense spectrographic bands of which cause a fog that obscures the "raie ultime" or sensitive line of lead at 4057.83 A.U. In consequence of this, the lower range of lead lines must be employed, *i.e.*, the "raie ultime" at 2833.07 A.U. and the minor lines at 2802.09 A.U. and 2614.20 A.U.

Under the conditions of our analysis it is convenient to compare the neighboring iron and lead lines, Pb 2833.07 A.U. and Fe 2832.54 A.U. In normal blood the iron line appears much denser than the lead line, while in lead poisoning Pb 2833.07 A.U. has an increased density which is about equal to or greater than the intensity of Fe 2832.54 A.U.

The initial smoldering and semi-charring process, which is in effect a partial micro-incineration, leaves a carbonaceous residue that does not burn as smoothly as a carbon-free ash, and occasionally results in a weak burn. Although this does not often happen when the electrodes have been properly prepared, as described above, it is to guard against such burn variation that three samples of blood are taken.

If a weak burn does occur, it can be detected easily by noting the general weakening of intensity of all the lines in the blood spectrum. This can be most readily appreciated by comparing the intensity of the lead line at 2833.07 A.U. with that of the neighboring iron line at 2832.54 A.U., as well as with the magnesium lines at 2802.71 A.U. and 2795.54 A.U. In a weak burn the iron line and the lead line are equally decreased in intensity, without any significant change in the

relative intensities of the two lines. The comparison of the lead line intensity with the iron line intensity thus forms a useful check on the mechanical variations inherent in the method.

In comparing bloods from different patients, one must bear in mind the variation in iron concentration due to varying degrees of anemia. For practical purposes, nevertheless, samples of blood from different cases may be conveniently compared in a similar manner because iron is a "spectrographically buffered" element. The individual intensity of any one of its numerous lines varies only slightly with comparatively larger variations in iron concentration.

Although the micro-method does not lend itself readily to quantitation, approximately quantitative estimations have been made by a simultaneous comparison with the results obtained by the ash method. In terms of absolute sensitivity, the micro-method permits the detection of as little as 0.00005 mg. of lead, or 0.05 mg. per 100 cc. of blood. However, the lead line at 2833.07 A.U. does not appear visible to the naked eye until the lead concentration rises above 0.1 mg. per 100 cc., that is, until it reaches the pathological range of approximately 0.2 to 1.0 mg. per 100 cc.

The method was originally intended for peripheral blood samples, but it can also be used with uncoagulated venous blood. In the case of the latter, of course, care must be taken to use a lead-free anticoagulant. In our experience it has appeared that unless a preliminary drying of the measured sample is resorted to, the uncoagulated venous blood is more difficult to burn than the quickly clotting peripheral sample.

DISCUSSION

During the past year we have tested this micro-method and have found it to be very useful in the rapid diagnosis of lead poisoning. The amount of blood necessary is very small and can easily be obtained from a finger puncture, a great advantage when dealing with small children. The detection of pathological blood lead, like the observation of the X-ray lead line in the bones of children (4, 5, 6), has been particularly valuable in differentiating lead encephalopathy from other possible causes of convulsions in patients.

A positive result indicates clinical lead poisoning, or an early stage

of convalescence from it. A negative result eliminates lead poisoning as a diagnostic consideration.

SUMMARY

1. A thirty minute spectrographic micro-method for the detection of pathological amounts of lead in 0.1 cc. samples of peripheral blood has been described.

2. It has been suggested that this rapid and convenient method may be a valuable aid in the diagnosis of lead poisoning.

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DEMONSTRATION OF ENCEPHALIC CONTROL OF MICTURITION BY ELECTRICAL STIMULATION

ORTHELLO R. LANGWORTHY AND LAWRENCE C. KOLB

From the Sub-Department of Neurology, the Johns Hopkins University

Submitted for publication November 12, 1934

INTRODUCTION

Voluntary micturition is developed during the early days of life and may be considered a conditioned reflex. Denney-Brown and Robertson (1933a), studying micturition in man, found that waves of contraction developed in the bladder wall when it was gradually distended with fluid. These waves increased in amplitude, and finally became fused into a constant tonic contraction which initiated emptying of the bladder. When these waves were well developed they could be made to cease completely by an effort of the will, while the intravesical pressure fell to a lower level. Denny-Brown and Robertson (1933b) later found that the power to stop the waves of contraction was lost in individuals with damage of the parasympathetic supply to the bladder due to injury of the spinal cord or of the nerves of the cauda equina. They concluded that the power to arrest bladder contractions was effected through the parasympathetic nerves, and also that the control of micturition by the cerebral cortex was entirely of the nature of inhibition of spinal reflexes.

In any study of the influence over micturition exerted by the cerebral cortex, it is impossible to think in terms of the parasympathetic or sympathetic pathways. Rather the total function of micturition is represented at this level. Damage to voluntary control may be expressed as inability to appreciate bladder distension, or to initiate or to stop micturition at will. Frequency may result from bilateral cerebral lesions (Langworthy and Kolb, 1933).

Holmes and Sargent (1913) studied 70 cases of thromboses of the superior longitudinal sinus; of these men 16 had incontinence as-

sociated with paraplegia and cortical anesthesia. Wilson (1923) reported the cases of two patients with cortical lesions who had difficulty of micturition. In one, recovery of the power of voluntary micturition occurred simultaneously with return of the power of movement of the muscles of the leg. Wilson concluded that the motor center for micturition was bilaterally represented, and lay in close relationship to the area controlling the muscles of the thigh. Kleist (1918) and Foerster (1918) found that there was a loss of voluntary influence over micturition after bilateral lesions of the paracentral lobules. Czyhlarz and Marburg (1901), from a study of patients with intracranial neoplasms, suggested representation of micturition in the cortex in the same area as that for the thigh musculature.

The experimental evidence of cortical representation of the function of micturition preceded by years the just mentioned clinical observations. Valentin in 1841 [quoted from Gruber, 1933] stimulated the cerebral hemispheres, crura cerebri, medulla oblongata, floor of the fourth ventricle, and the spinal cord in the rabbit, and observed contractions of the bladder. Budge (1864) supported these observations on stimulation of the cerebral peduncles, corpus restiforme, and calamus scriptorius. Karpus and Kreidl (1909) noted, after stimulation of the corpus Luysi, the contraction of the urinary bladder. Lichtenstern (1912) postulated a center for micturition in the hypothalamic region where he obtained strong vesical contraction following electrical stimulation. Spiegel and MacPherson (1925) obtained bladder contractions on stimulation of the cerebral peduncles. Spiegel and Tokano (1928) found that no vesical contraction occurred on stimulation of the hypothalamus or corpus Luysi, if the motor cortices had previously been removed and the fibers of the internal capsule allowed to degenerate. Since the fibers of the internal capsule pass through this region, it is not possible normally to stimulate the corpus Luysi and hypothalamus without exciting the fibers of the internal capsule.

The relations of the mid-brain and medulla to micturition are of particular interest. Barrington (1921) observed that in cats the bladder never emptied itself completely after there had been a lesion of the upper portion of the spinal cord, and that residual urine was

constant. He localized bilateral areas in the mid-brain, where injuries affected the bladder so that it no longer emptied itself entirely. He noted further that bladder contractions were obtained on electrical stimulation of the mesencephalon about 2 mm. from the midline just ventral to the caudal end of the aqueduct.

We ourselves (Langworthy and Kolb, 1933) reached somewhat different conclusions concerning the function of the mid-brain in micturition. After decerebration through the optic colliculi, the bladder emptied itself reflexly on as little as one-fourteenth the volume required to produce reflex micturition in the normal anesthetized subject. Transection of the brain stem below the acoustic colliculi abolished the reflex, so that no contraction sufficient to empty the bladder occurred; the condition of retention with overflow was present. The conclusion was reached that tone in the musculature of the bladder is controlled by mechanisms similar to those which control tone in striated musculature. Some reduction of volume followed removal of the cerebral motor areas, so that there was evidence of the control of the tonic mechanism from the cerebral cortex.

The pathways for micturition in the spinal cord were studied by Budge (1864) who stated that, after removal of the dorsal half of the spinal cord, stimulation of the ventral half evoked contractions of the bladder. However, Stewart (1899) found that impulses controlling emptying of the bladder traversed the lateral columns of the cord, and crossed the mid-line from the second to the fifth lumbar segments. He also noted some decussation of the fibers in the lower portion of the medulla and the first cervical segment. Barrington (1933) stated that both the centrifugal and centripetal paths for micturition are situated in the dorsal half of the lateral column near its periphery.

METHODS

Thirty-one female cats were used in the experiments. Anesthesia was induced by the intraperitoneal injection of nembutal, 0.5 cc. per kilo of body weight. This produced a sufficiently satisfactory depth of anesthesia for the cranial operation, and to maintain a relaxed position of the animal without affecting the cortical responses to electrical stimulation.

In preparation for the craniotomy a vertical incision was made in the midline of the neck, the common carotid arteries located, separated from the vagus nerves, and then tied. A tracheal cannula was next inserted, and attached to a Harvard artificial respiration pump which was run constantly. We feel that the use of this respirator, causing continued oxygenation of the blood, was most effective in preventing asphyxia which was likely to occur after sections made through the rhombencephalon. The entire calvarium was next removed: bleeding was controlled. The dura was incised bilaterally over the motor cortices, and the cortex was dried with cotton pledgets. Stimulation was effected with a stigmatic Sherrington electrode, a platinum point mounted on a delicate spring. The large indifferent electrode was moistened and applied to a shaved area on the lateral abdominal wall. The electrodes were connected with a Harvard inductorium.

A fine glass catheter was then passed into the bladder through the urethra, and the free end attached to a recording apparatus. The catheter was not tied into the bladder but was held in position by the tone of the sphincters. With the onset of micturition, the fluid was able to escape freely about the catheter which then became loose in the urethra. The catheter was connected with a three-way tube; the other arms were attached to an open-end water manometer of 3 mm. bore, and a burette graduated to one-tenth of a cubic centimeter. By means of the burette, water warmed to body temperature could be introduced into the bladder when necessary. The intravesical changes of pressure were rapidly reflected in our manometer.

The cortex was usually stimulated, then a transverse section of the brain removed the motor areas: the cut surface was dried, and the electrical exploration of the face of this section was made. Further sectionings were performed at successively caudal levels, so that in some instances the stimulation was continued to the lower medulla or upper cervical cord.

EXPERIMENTAL RESULTS

1. Stimulation of the cerebral cortex

The areas from which responses were obtained on stimulation of the cerebral cortex will be described as plotted on the diagram (fig. 1).

The electrically responsive motor area surrounds the cruciate sulcus, and many of the large pyramidal cells are buried in the sulcus. Previous experiments (Weed and Langworthy, 1926) have shown that stimulation of Areas A and B anterior to the sulcus elicits contractions of the contralateral fore-leg musculature; stimulation of Areas E and F posterior to the sulcus gives rise to contractions of the con-

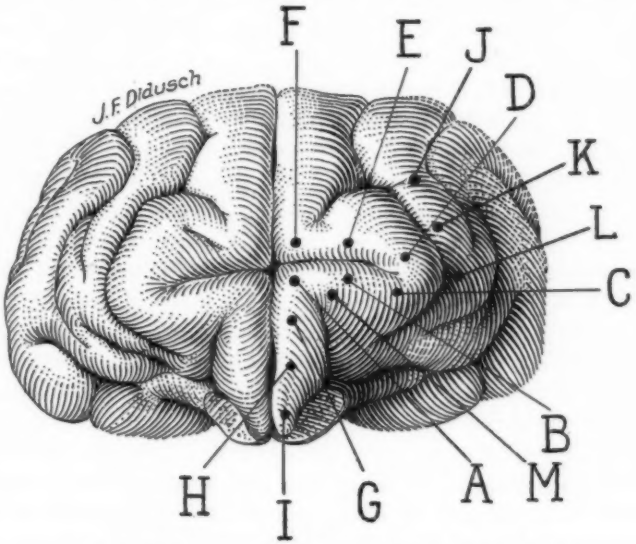


FIG. 1. THE CEREBRAL MOTOR CORTEX OF THE CAT

After a considerable latent period, rises of bladder pressure and micturition could be elicited by stimulation of Area M. If the bladder pressure was high, stimulation of this area would cause the pressure to fall.

Rises of bladder pressure were also obtained from Area E and the cortex between Areas E and F. These rises occurred at once following stimulation.

tralateral hind-leg. Movements of the vibrissae and masticatory muscles are usually obtained from Area C. Anterior to this region is the premotor cortex which contains smaller pyramidal cells and has no granular layer (Langworthy, 1928). Cortex of this type is present in Areas A, G and M.

The most marked responses of the bladder on stimulation of the

cortex were obtained from Area M. This area is not contiguous to the region which gives responses of the contralateral hind-leg, but rather lies in the premotor cortex, anterior to the area which effects movements of the contralateral fore-leg. If any responses of striated muscle were observed simultaneously with changes in the bladder pressure on stimulation of Area M, they were movements of the contralateral fore-leg. It was thought that these fore-leg movements were due to the spread of the current to the adjacent Areas A and B.

The responses in the bladder did not occur immediately on stimulation of Area M. There was usually a latent period of as long as thirty seconds before any rise of pressure took place. Often it was noticed that the pressure did not rise until the electrode was removed; it then increased very rapidly, and led to the emptying of the bladder.

The extent of the pressure rise varied in different preparations. In many cases the pressure reached 40 cm. of water, and fluid escaped from the bladder. At other times a moderate increase from 6 cm. to 20 cm. of pressure was observed, and the pressure returned almost at once to 6 cm. The changes induced in the bladder by stimulation were dependent primarily on the irritability of the muscle. The most striking results were seen when the bladder was filled almost to the limits of its capacity. After stimulation had produced emptying once, and the bladder was then refilled with water, micturition could be induced more easily than before. The most advantageous preparations were those in which moderate waves of contraction were constantly seen in the manometer before stimulation was begun. The pressure increased on stimulation to a certain height at a moderate rate. Then the rate of rise was accelerated, and became independent of stimulation so that the rise continued even after the electrode was removed. Water then flowed from the urethra.

After a marked increase in it had been produced by prolonged stimulation, the bladder pressure often remained high, between 30 and 40 cm. of water, for a considerable length of time. It was found that stimulation of Area M on the side which had not been previously excited, caused the pressure to fall at once to normal. After a latent period a fall could be obtained on stimulation of the same area that gave the initial rise.

Stimulation of Area E, or the region between Areas E and F, raised

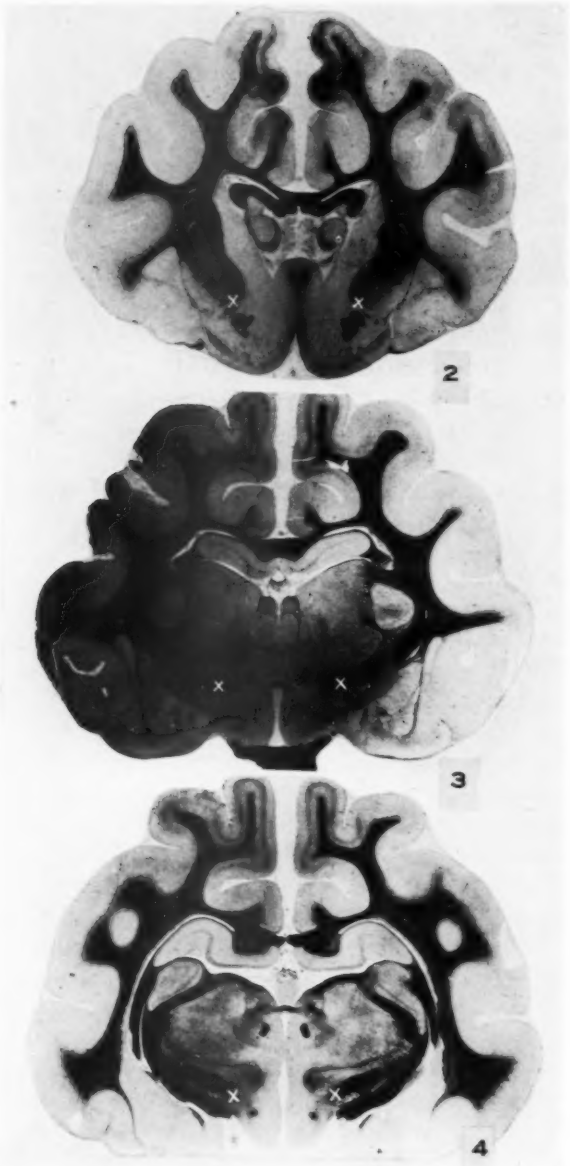
the pressure in the bladder; the response was quite different from that obtained from Area M. After the electrode was placed on Area E, a quick rise of intravesical pressure occurred, increasing from 6 to 14 cm. of water. This did not lead to micturition, and the pressure fell rapidly again to normal. Following the elevation of pressure, movements of the contralateral hind-leg were obtained. The bladder contraction in this case had a shorter latent period than that for striated muscle.

It appeared that more marked responses could be obtained from one motor cortex than from the other; the left cortex usually gave the greatest changes in vesical pressure on stimulation of Area M. In some cases mechanical stimulation of the cortex by drying with cotton caused the bladder pressure to rise.

2. Stimulation of transverse sections of the brain stem and cervical cord

Not infrequently when the knife was passing through the ventral portion of the forebrain, the bladder pressure rose, and occasionally the bladder emptied itself. Micturition was almost certain to take place when the brain stem was sectioned. The first section traversed the caudate nucleus (fig. 2), and the second the thalamus (fig. 3). At both levels a change in bladder pressure was obtained from stimulation of the ventro-mesial portion of the internal capsule from the areas marked in the photographs. The latent period was considerably shorter than on stimulation of the cortex, so that almost at once the pressure rose, and usually micturition began. If the pressure remained high after this stimulation, it fell immediately on excitation of the corresponding area on the contralateral side. A similar result was obtained from stimulation of the first area if a short period of time was allowed to elapse between applications of the electrode.

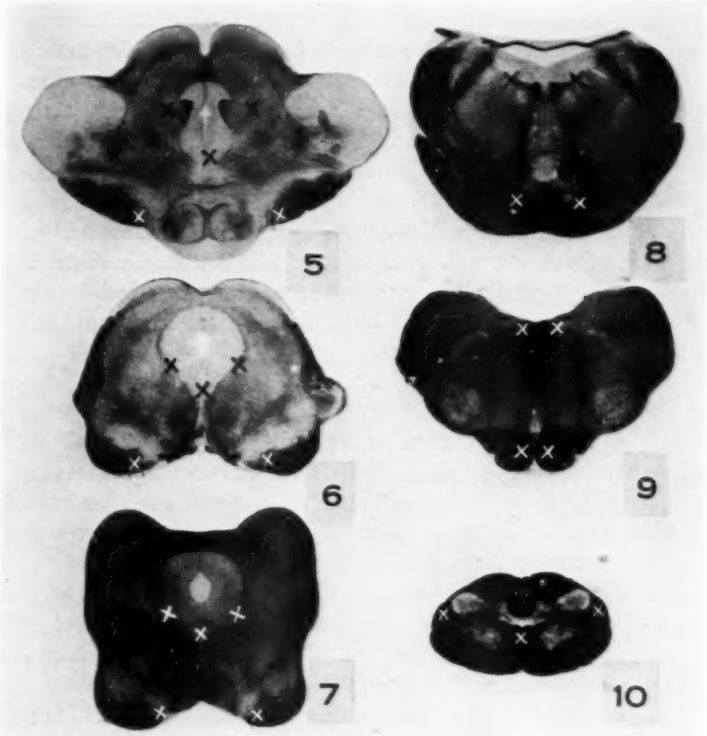
Other regions giving bladder responses on electrical stimulation were first observed after sectionings which cut through the posterior commissure (fig. 4). Stimulation of areas just lateral to the posterior commissure gave rise to increased bladder pressure and micturition. Responses from this region were even more effective in causing elevation of intravesical pressure than were those from the internal capsule. The latent period was extremely short. No fall of pressure could be produced by stimulation of the area on the opposite side.



FIGS. 2 TO 4. TRANSVERSE SECTIONS OF THE CAT'S FORE-BRAIN

Changes in bladder pressure were obtained on stimulation of the areas which are marked on the photographs.

The responsive areas in the internal capsule could be followed through the midbrain and medulla into the lateral columns of the spinal cord (figs. 4 to 10). At the level of the decussation of the pyramids (fig. 10) responses were obtained from the midline. The



FIGS. 5 TO 10. TRANSVERSE SECTIONS OF THE BRAIN STEM OF THE CAT

Changes in bladder pressure were obtained on stimulation of the areas which are marked on the photographs.

results of stimulation below the level of the pons were satisfactory in only a few cases; changes obtained from the pyramidal tracts below this level were similar to those elicited from Area E of the cerebral

cortex, but were not so well sustained as those obtained from Area M or from the mesial portion of the internal capsule above the level of the pons.

Responses from the dorsal portion of the brain stem on stimulation of sections caudal to the posterior commissure were regularly obtained. At the upper end of the midbrain (fig. 5) a rise of bladder pressure was elicited lateral to the aqueduct of Sylvius, and also from the midline ventral to the aqueduct, suggesting that the fibers cross at this level. The same results were found from a sectioning through the optic colliculi, as indicated in figure 6. At the level of the acoustic colliculi (fig. 7), the responsive areas were ventral to the aqueduct, and also in the midline.

Figure 8 illustrates a section at the level of the trochlear nerve and the pons. Stimulation of an area ventral to the fourth ventricle in the region of the lateral fibers of the medial longitudinal fasciculus elicited a rise of pressure in the bladder. Similar results were gained at lower levels in the medulla (fig. 9).

DISCUSSION

Localization of the control of micturition in the cerebral cortex seems evident from these experiments, and from the other observations heretofore mentioned. A single coordinated cerebral mechanism may act at times to decrease vesical pressure or may elevate it to a point where micturition takes place.

This seems to be supported by the fact that stimulation of an area, just anterior to that which gives responses from the striated musculature, effected various changes in the pressure of the bladder contents. Increases in pressure were obtained by continued stimulation of this area from which pressure decreases were also elicited once the rise had commenced.

No increases in vesical pressure were obtained from the hypothalamus or corpus Luysi, but only from the mesial portion of the internal capsule. We feel, with Spiegel, that such responses which were obtained by other workers resulted from the spread to the fibers of the internal capsule. We ourselves stimulated the internal capsule in two cases where the motor fibers were degenerated and obtained no responses.

These experiments demonstrate by another method the previous findings of Barrington (1921 and 1925) and of Langworthy and Kolb (1933) that there is a midbrain factor in the control of micturition. This control can first be noted at the level of the nuclei of the posterior commissure. The fibers apparently cross the midline in the rostral end of the midbrain to run downward just ventral to the fourth ventricle in the most lateral portion of the medial longitudinal fasciculus.

The authors believe that this midbrain mechanism controls the tone in the bladder musculature. Richter and Langworthy (1933) using the smooth muscle of the porcupine's quills for a study of autonomic control by the brain found that the quills were made to stand on end when a sectioning of the brain stem was made at the level of the posterior commissure. It is probable that there is in the midbrain a control of tone of smooth muscle throughout the body.

Denny-Brown and Robertson (1933b) have objected to Barrington's suggestion that there is a midbrain control of micturition, feeling that his findings were dependent on the development of shock. The present experiments dealing with stimulation of the midbrain appear to dispose definitely of this objection inasmuch as these clear-cut rises of pressure could not be obtained on stimulation unless there was an intact pathway from the dorsal portion of the midbrain which would influence the bladder musculature. Moreover, it is universally agreed that animals with sections above the brain stem do not show spinal shock.

There is, then, in the region of the cerebral motor cortex a mechanism which controls the function of micturition as a whole. Similarly, in the midbrain there is a mechanism having to do with tone in the musculature of the urinary bladder. The level of correlation of these two influences is not clear. It is perhaps most logical to believe that the final integration takes place at the level of the preganglionic autonomic cells in the spinal cord. It is possible, however, that the cortex exerts a control over the midbrain mechanism either through fibers passing directly to the cells concerned or through the agency of the cerebellum.

SUMMARY

Electrical stimulation of an area in the premotor cortex of the cat produces changes in bladder pressure. After a long latent period the pressure rises and micturition occurs. Stimulation of this same area causes the bladder pressure to fall when it is already high. A small and poorly sustained rise occurs on excitation of the area from which contralateral hind-leg movements can be elicited.

Stimulation of an area lateral to the posterior commissure or near the aqueduct of Sylvius in the midbrain effects elevation of bladder pressure and micturition. This paper furnishes further proof of a midbrain control of tone in the urinary bladder.

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THE IRON OF THE BLOOD

A Comparison of Values for Haemoglobin Determined by the Newcomer Method and Calculated from the Iron Content[1]

HUGH W. JOSEPHS

From the Harriet Lane Home of the Johns Hopkins Hospital, and the Department of Pediatrics, Johns Hopkins University

In recent years there has appeared a tendency to use the determination of the iron content of the blood as a means of estimating the haemoglobin. Van Slyke and Peters (1) give a method for iron determination along with the methods for estimating haemoglobin, and state that this method can be used for standardizing the colorimetric procedures. There are a number of articles in the literature in which in the so-called "normal" comparison has been made between oxygen capacity, iron content, and acid haematin determination of haemoglobin (2). In general, agreement has been found between these three methods in the normal case. Since the acid haematin method is the one most widely used clinically and the one most likely to be continued in use, it seemed worthwhile to compare the haemoglobin values obtained by the Newcomer method with those calculated from the iron content of the whole blood, in subjects other than normal.

METHODS

Iron determination was made on the whole blood as follows: 0.1 cc. blood from a stab in the heel was drawn into a capillary pipette and delivered into a few cubic centimeters of distilled water. This was later transferred to a 50 cc. Kjeldahl flask. One cubic centimeter of concentrated sulphuric acid and 0.5 cc. perchloric acid were added and the mixture ashed. The iron was then determined colorimetrically by the thiocyanate-amyl alcohol method essentially as described by Kennedy. Haemoglobin was determined by the Newcomer method,

[1] Submitted for publication November 23, 1934.

using blood from the same stab, which was made deep enough to obtain sufficient blood without the necessity of squeezing.

In an interpretation of the results, account must be taken of the error of the methods. This has been assumed not to exceed ± 5 per cent for both the iron determination and the haemoglobin determination. The combined errors would then be about ± 10 per cent.

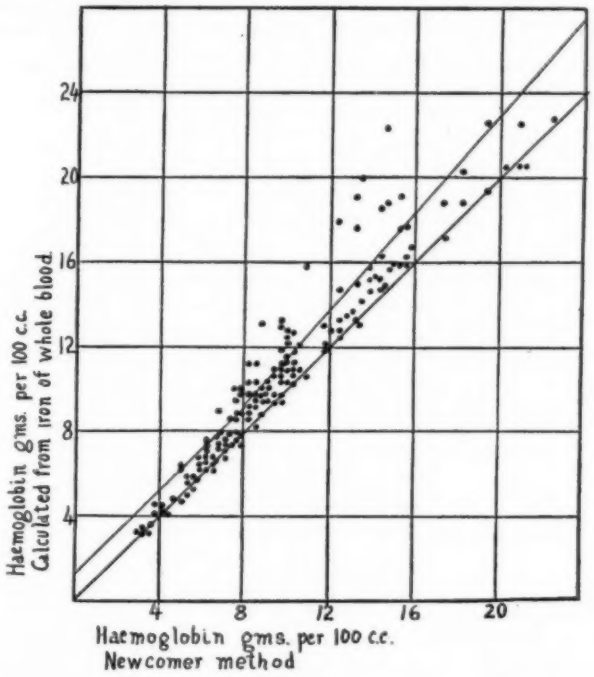

CHART 1

Actually the error is probably smaller than this, but it seemed best to allow for an error that would satisfy the most critical and to treat as abnormal only those figures that lay outside the range of this error. In cases in which the amount of iron in the 0.1 cc. sample was below .03 mg., the error was taken as .0015 mg., so that for such cases the total error would exceed 10 per cent, the percentage error increasing

as the amount of iron in the sample decreases. This figure is admittedly arbitrary but errs on the side of conservatism.

In Chart 1 the haemoglobin calculated from the iron determination $\frac{(\text{mg. Fe in the sample})^2}{.0034}$ has been plotted as ordinate against the haemoglobin determined by the Newcomer method as abscissa. The lower of the two diagonal lines denotes agreement between the two methods. As may be seen the haemoglobin calculated from the iron content of the whole blood tends to be higher than that deter-

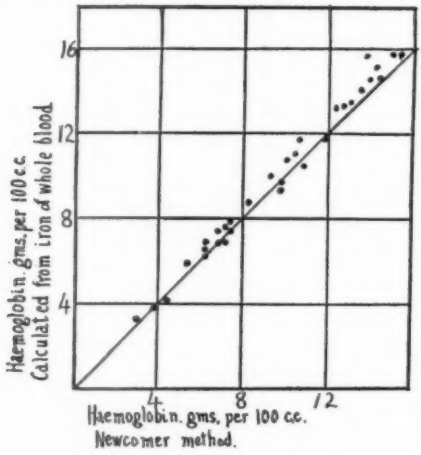

CHART 2

mined by the Newcomer method, in some cases markedly so. The second of the two lines denotes a 15 per cent deviation from the expected agreement. Points beyond this line were considered definitely abnormal. The cases from which these abnormal points were obtained fall into three groups: (1) infants between birth and eight weeks of age, both those born prematurely and those born at term; (2) children with infection (two cases); (3) anaemic infants receiving iron with or without copper supplement.

These cases are, then, the ones in which one may expect an ab-

[2] The figure for iron content of haemoglobin, as based on the work of Butterfield and of Peters (3) which is now generally accepted, is 0.34 per cent.

normally high iron content in the blood. Excluding these, we have left a group in which the normal relationship between iron and haemoglobin may be expected. The points obtained in these "normal" cases are plotted in Chart 2. The cases include normal children, a few normal adults, infants and children convalescent from infection, infants after the tenth week of life in the hospital for study of phases of normal metabolism and infants with anaemia not receiving iron.

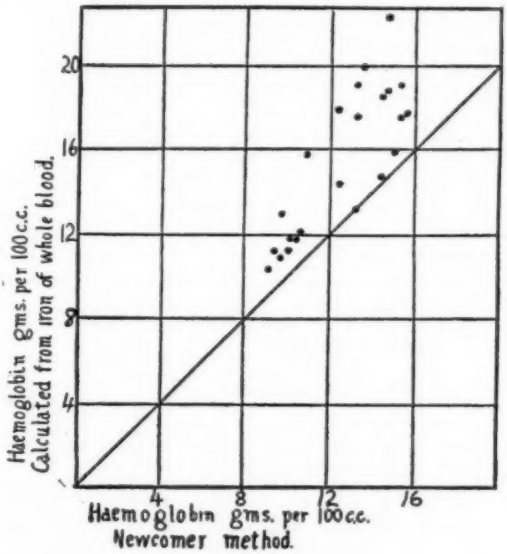

CHART 3

Only one point deviates from the expected more than the 10 per cent set as the upper limit of error. It may be seen, however, that the average of the points lies slightly above the line denoting the expected relationship. This would mean that normally there is iron present in the blood, not accounted for by the haemoglobin if we accept the Newcomer method as standard. This extra iron amounts to about 3 mg. per 100 cc., therefore more than can be accounted for by the iron of the plasma.

In Chart 3 are plotted the determinations made on full-term infants

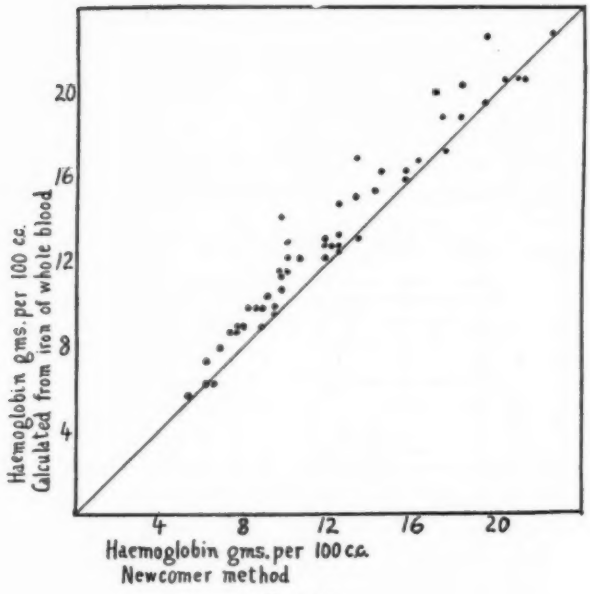

CHART 4

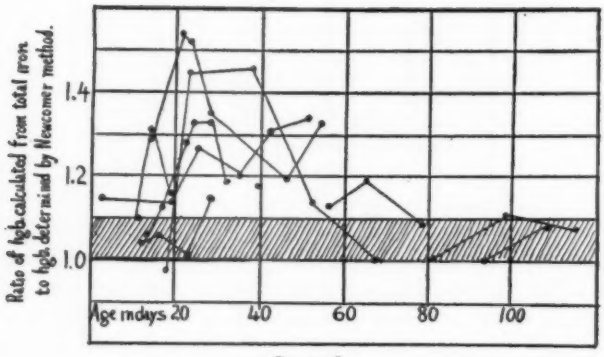

CHART 5

during the first ten weeks of life, and in Chart 4 those made on premature infants during the same period. As may be seen, the haemoglobin calculated from the iron content of the blood is in many cases much higher than that determined by the Newcomer method.

In Chart 5 the ratio of the haemoglobin calculated from the iron to that determined by the Newcomer method has been plotted against the age of the infant in days. From this chart it is seen that the ratio rises sharply at about two to three weeks of age and, after reaching

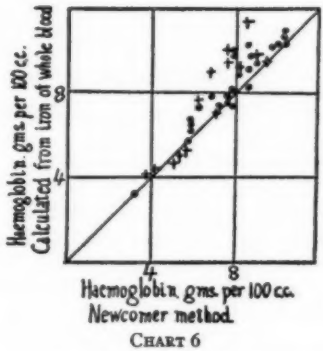

CHART 6

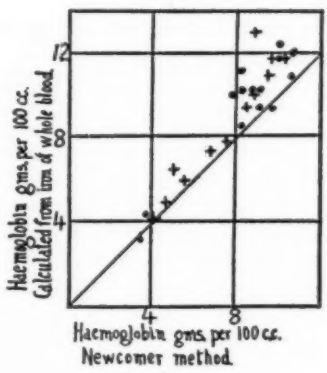

CHART 7

its height, returns to within normal limits at about the eighth to tenth week of life.

Chart 6 contains the observations on cases of anaemia receiving iron and Chart 7 those on cases receiving iron and copper. The points refer to determinations made while the haemoglobin was stationary, the crosses to determinations made while the haemoglobin was rising.

No discussion of these figures is possible at the present time. It is merely desired to point out that under certain conditions there is much more iron in the blood than can be accounted for by the haemoglobin as determined by an acid haematin method. The determination of what function this iron has, if it has any at all, must await future work.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of Editorial board of this journal.)

Who Shall Survive? A New Approach to the Problem of Human Interrelations. By J. L. MORENO. 440 pp. (*Nervous and Mental Disease Publishing Co., Wash., D. C., 1934.*)

Dr. Moreno's book is one of the valuable monographs on Nervous and Mental Diseases published by the company that has already given us the works of Dr. William A. White. It will probably be far beyond the grasp of any layman, who knows little or nothing about modern sociology and the progress of its investigations. The book is really a study of the interaction of individuals in a small group and the reaction of that group on other groups. Dr. White himself introduces us to Dr. Moreno's work in a very able preface. The book is not easy reading, but will be of interest to all teachers and to psychiatrists.

J. R. O.

Clio Medica. Japanese Medicine. By Y. FUJIKAWA. Translated from the German by JOHN RUHRÄH, with a chapter on the Recent History of Medicine in Japan by KAGEYAS W. AMANO. 8 illust. 114 pp. $1.50. (*Paul B. Hoeber, Inc., New York, 1934.*)

This is one of a valuable series of Primers on the History of Medicine of which ten or twelve volumes have already been published. Our own Dr. Ruhräh has translated from the German this short history of Japanese Medicine by Dr. Y. Fujikawa, and Dr. Amano, a Japanese physician, has brought this history up-to-date. The book has a very useful chronological table and good indices.

J. R. O.

Clio Medica. Chinese Medicine. By WILLIAM R. MORSE. 6 illust. 185 pp. $2.50. (*Paul B. Hoeber, Inc., New York, 1934.*)

Dr. William R. Morse, Dean of the Medical School in West China Union University, has prepared the volume on Chinese Medicine. Dr. Morse feels that the Chinese have emphasized, perhaps over-emphasized, a principle that our European Medicine has denied and lost. For Chinese Medicine, at its best, is vitalistic, while ours is materialistic (p. 166). Dr. Morse gives a list of useful references for supplementary reading.

J. R. O.

Clio Medica. German Medicine. By W. HABERLING. Translated by JULES FREUND. 9 illust. 160 pp. $1.50. (*Paul B. Hoeber, Inc., New York, 1934.*)

Professor Haberling's brief history of German Medicine has been translated by Dr. Jules Freund. Dr. Haberling is Professor of the History of Medicine in Düsseldorf. His primer contains all the great names and the outstanding works of the great men of medical Germany. One notes an occasional misprint, *e.g.* (p. 16), Coranus for Cornarius. Otherwise the book is adequate and interesting.

J. R. O.

Textbook of Materia Medica and Therapeutics. By A. S. BLUMGARTEN. 6th edit. 791 pp. $3.00. (*Macmillan Co., New York, 1934.*)

The appearance of a 6th edition of this book is ample evidence of its success as a text for student nurses, for whom it is primarily intended. In the present edition the subject matter has been completely revised and brought up-to-date. The book is unfortunately marred by statements such as the following: "Citric acid is frequently given on shipboard as an article of diet to prevent scurvy, a severe disease of the joints due to the lack of vitamines . . ." (p. 181). "Histamine is a chemical substance (amine formed from amino acids, the end substance of protein digestion" (p. 202). "The normal amount of calcium in the blood varies from 9 to 11 milligrams in 1000 cc., of which 6 milligrams are combined as diffusible calcium and about 2 milligrams exist as ions" (p. 273).

A. G.

Alcohol and Man. The Effects of Alcohol on Man in Health and Disease. HAVEN EMERSON [Editor]. 451 pp. $3.50. (*Macmillan Co., New York, 1932.*)

This book, containing a number of separate papers contributed by biologists, pharmacologists, clinicians and others, is intended primarily for the laity. The general presentation of the subject is fair and well-balanced. The authors have avoided all implications of "dryness" or of "wetness," but the general impression left on the reader is that "Wine is a mocker" and that the less gin one drinks the better.

J. R. O.

Applied Physiology. By SAMSON WRIGHT. 5th edition. 604 pp. (*Oxford University Press, New York, 1934.*)

In the present edition this well known book has been largely rewritten and revised, with new illustrations. Passages of merely clinical interest are printed in italics, so that they may be omitted by any student who is reviewing pure physiology.

J. R. O.

The Autobiography of a Suicide. Anonymous. Psychology Guild Selection. 223 pp. (*Lawrence, Long Island, Golden Galleon Press, 1934.*)

The author is said to have been a writer of distinction, an editor and a prominent newspaper man. All his life he suffered from fear reactions of various types, which he describes in detail, leaving himself in the last chapter just ready to eliminate himself from the earth. His past career certainly had had little influence on the clarity of his English, which is as sloppy as his emotions. The book is pathetic, because the writer's mind has become so concentrated upon himself that he has never been able to see himself in proper perspective and to realize how unimportant his sufferings really were. It seems impossible to believe that he could ever have been cured, even by the eminent psychiatrists to whom his book is dedicated. One is almost forced to conclude that he did not want to get well. There are thousands of fear-ridden people who do not surrender to their fears as easily as the writer of this book.

J. R. O.

De Venarum Ostiolis. 1603 of Hieronymus Fabricius of Aquapendente, (1533?–1619). Facsimile edition with introduction, translation and notes by K. J. Franklin. 1933. 98 pp. (*Chas. C. Thomas, Springfield, Ill. & Baltimore, Md. $3.00.*)

Fabricius ab Aquapendente was not the first discoverer of the venous valves, but it was due to his work that the existence of such valves became generally known to the physicians at the beginning of the 17th century. And despite the fact that Fabricius himself still tried to explain the functions of the valves in accordance with the traditional Galenic physiology, his contribution was of the greatest importance for the later discovery of the circulation of the blood by his pupil William Harvey.

In the present book, Dr. Franklin gives not only a facsimile edition and English translation of the "De venarum ostiolis" but he also deals with Fabricius's biography, the early history of the venous valves, the anatomical theatre in Padua and the bibliography of the first edition. Thus he enables the reader to read the original work itself, and at the same time to see it under the historical perspective which connects it with the past and the future.

O. T.

The History and Epidemiology of Syphilis. By Wm. Allen Pusey. 113 pp. $3.00. (*C. C. Thomas, Springfield and Baltimore, Md. 1933.*)

This book comprises lectures delivered under the Gehrmann endowment at the College of Medicine, University of Illinois [Chicago] on May 1–3, 1933. Lectures I and II [74 pages] are an understandable and informing history of syphilis, covering all the essential facts. The Sydenham-Sudhoff view of the tropical [pre-Columbian] origin of European syphilis is combated on the ground that the medi-

eval expressions *gros mal* and *petit mal* referred to epilepsy in the first instance. Lecture III is epidemiological. The writer concludes that control of syphilis is a sanitary [non-medical] problem. The illustrations are instructive and there is a good terminal index.

F. H. G.

The Francis Treatment of Asthma. By ALEXANDER FRANCIS. 160 pp. 7/6. (*William Heinemann, Ltd., London, 1933.*)

A book on the author's method of treating asthma by nasal cauterization which he has employed with success for thirty years.

E. M. B.

EFFECT OF ALCOHOL ON CORTICAL AND SUBCORTICAL ACTIVITY MEASURED BY THE CONDITIONED REFLEX METHOD

W. HORSLEY GANTT, M.D.

From the Conditioned Reflex Laboratory of the Phipps Psychiatric Clinic, Johns Hopkins University

Submitted for publication November 24, 1934

In spite of the volumes that have been written on the pharmacological and psychological action of the alcoholic beverages, there still seems to be a place for the quantitative determination of their effect on behavior. Our present work has no bearing on the many social and biological aspects of the alcohol problem, nor on influences that may be built around the various associations connected with its use by man. The whole psychobiological importance of alcohol is too complicated to be solved by a simple laboratory experiment, and from general experience we already have more information on that aspect than we can apply.

In the present experiments we attempted to find out whether alcohol stimulated or depressed cortical activity; whether it added to the intensity or speed of conditioned responses, both motor and salivary; what was its effect on the unconditioned food (salivary) reflex, and finally, what was its relative influence on the processes of excitation and inhibition on which is based differentiation or discrimination.

We studied its action on five dogs, all of whom had been in the laboratory for a year or more and who had well-established conditioned reflexes. Following is a description of the animals: *Kompa* was a female in whom we first elaborated conditioned reflexes in 1930, and then produced sleep by their extinction. She was excitable and rather unstable. She developed a differentiation between two metronoms (M160 and M60) of 160 and 60 beats per minute with some difficulty. For 2 years previous to the alcohol experiments she had had a regular daily routine of 7 conditioned stimuli, the second and sixth of which were inhibitory. Four other dogs, *Lady*, *Billy*, *Blue*,

and *No. 3* were employed for the elaboration of conditioned auditory reflexes connected with different amounts of food—a problem involving differentiation to intensity of unconditioned stimulus (different amounts of food evoking different degrees of excitation) rather than absolute differentiation (food and no food, excitation and inhibition) as in Kompa. The two conditioned signals used for all these 4 animals were a metronom of 20 beats per minute (M20) and a sound of air bubbling through water (BU). In Blue and Billy, the BU was the signal for the large amount of food (18 grams of dry dog biscuits) and M20 for the small amount of food (3 grams), whereas in Lady and No. 3 BU was the signal for the small amount of food and M20 for the large. No. 3 might be considered analogous to an oligophrenic, as he had had most of the left cortex removed in 1931, a year before these experiments, and he elaborated the reflexes and differentiation with difficulty. Convulsions from the cortical lesion occurred from time to time, and he died in August, 1933. Billy was a stable male American Pitt bull terrier, weight about 16 kgm., aged about 4 years; Lady was a female of the same breed and size, aged about 2 years; Blue, a male, timid collie, aged $1\frac{1}{2}$ years, weight 18 kgm.; No. 3, a female hound, aged about 3 years, weight 10 kgm.

ADMINISTRATION

Twice at the beginning of the experiments the alcohol was given by mouth through a stomach tube. Although this procedure apparently had no influence on the conditioned reflexes in the two experiments in which it was used, we abandoned it, because we found that the animals could be induced to drink the alcohol if it were not stronger than 20 to 25 per cent. Accordingly, ethyl alcohol, 95 per cent, was mixed with an equal amount of milk and diluted with water to make a solution of 20 per cent alcohol. Lady would drink the alcohol, as a rule, without the addition of milk. The others had to be coaxed by putting crackers into the milk-alcohol mixture. After the first few times the animals drank it more readily. The mixture had a sharp alcoholic odor, and the dogs apparently objected to this more than to the taste. They would walk in circles around the pan, sniffing, until one or two crackers were dropped into it, but when once they started, they would drink readily. No. 3 often refused

altogether unless the alcohol was diluted to 10 per cent. The large amounts were taken as readily as the small quantities, provided that the dilutions were the same. Three different-sized doses were given to most of the animals: 0.5 cc. alcohol per kilogram body weight, 1.5 cc. alcohol per kilogram, and 3.0 to 4.0 cc. alcohol per kilogram. Thus the small doses would be equivalent to an ordinary tumblerful or to half a pint of port wine for a man of 150 pounds or half this amount of whisky, and the other doses were three and six times as much.

The animals were put into the experimental *camera silenta* 10 to 30 minutes after the ingestion of the alcohol and the conditioned and unconditioned responses were recorded for about an hour. Thus the time of the maximum effect of the alcohol was included, since it has been shown that the highest concentration in the blood occurs 60 to 90 minutes after ingestion. The solution was given on an empty stomach, the animals receiving their daily feeding after the experiment.

Control experiments were done on the intervening days. Ordinarily, two alcohols and two controls were done in a week, only one experiment each day. As conditioned and unconditioned reflexes vary somewhat from day to day, depending on the appetite, health, and general condition of the animal, the question arose of performing the control immediately before the alcohol experiment and this was done in the case of the large doses. However, with the other doses, it seemed better to do controls on separate days, and to eliminate chance fluctuations by using a larger number of experiments. The most important disadvantage of doing two experiments on the same day is that the hunger of the animal diminishes with each feeding, and the reflexes may gradually fall in intensity after an hour of experimentation, and the latent period increases so that the measurements here would be misleading. On the control days the same quantity of milk and water was fed before the experiment as on the alcohol days.

The animal was allowed to run out of the room after taking the alcohol, and its general behavior was observed during this time as well as immediately after it was brought out of the *camera silenta* at the end of the experiment.

In Kompa, who gave a copious secretion, the conditioned reflexes were recorded for a period of 10 seconds, but in the other dogs one

30-second reading was taken daily, as is shown in the protocols. The unconditioned reflexes were always measured for 60 seconds, a time that includes practically the whole of the unconditioned salivary secretion to the amount of food given.

1. Effect of small doses (0.5 cc. per kilogram)

With doses of 0.5 cc. per kilogram diluted to 20 per cent we noted no effect on the ordinary behavior of the three animals (Kompa, Billy, and No. 3). They played as usual, running about the room without change in gait, jumping for food without loss of balance, showing no ataxia. Observations were made for one or two hours after ingestion. The dogs responded to the experimenter in the usual manner.

Alcohol experiments with the above dose were done on Kompa on March 7, 9 24, 28; November 15, 17, 21, 23, 1932, with controls on the intervening days. There was an increase in the latent period, both motor and secretory, and a decrease to 85 per cent in the 60-second unconditioned salivary reflex. Differentiation between two metronoms, relatively easy for most animals but accomplished with difficulty by Kompa, did not suffer, and though no better than in the controls, it was surprisingly good during the period of our experiments.

No. 3, the animal with a damaged cortex, whose learning capacity was below normal when judged both by the speed of formation and the stability of the conditioned reflexes, was tested with alcohol experiments on November 18 and 25, 1932, with controls on the intervening days.

Experiments with small doses on Billy were done on February 14, 16, 27, on March 1, 6, 8, and 12, 1934; control experiments on the intervening days. The 30-second reading of the stronger conditioned reflex was reduced by alcohol to 98 per cent of its control value, the 10-second reading to 90 per cent; the weaker of the two reflexes was reduced to 86 per cent of its normal value for 30 seconds, and to 69 per cent in the 10-second reading. The two unconditioned reflexes to the large and small amounts of food were reduced in about equal amount, to 95 and 96 per cent, respectively. There was a slight increase in both the motor and secretory latent period for the weaker

conditioned stimulus, but a reduction of the secretory latent period to 90 per cent with the stronger stimulus.

In Billy and No. 3 we see a more marked effect of the alcohol on the weaker of the two conditioned reflexes, not only on the absolute value of the secretion but also on the motor and secretory latent periods. In No. 3 there was a complete loss of the conditioned reflex to the weaker conditioned stimulus. In Lady, the reverse was true.

2. Moderately large doses

Three times the quantity of the former dose, *i.e.*, 1.5 cc. per kilogram, was given to 4 dogs, Kompa, Billy, Blue, Lady, on the following dates:

Kompa on February 15, March 30, April 4, 6, 8, 14, 18, 20, 26, May 2, 5, 10, 1932.

Billy on March 24, 28, 30, April 4, 6, 20, May 2, 5, 1932.

Lady on March 4, 31, April 4, 13, 15, May 3, 9, 1932.

Blue on March 29, 31, April 5, 7, 11, 14, 18, 25, 28, May 5, 1932.

An equal number of controls were done on the intervening days.

Effect on external behavior. Kompa in the first experiment showed some incoördination and ataxia before she was put in the camera, and unsteadiness on the table during the hour when the experiment was being done; in subsequent experiments nothing unusual was noticed except perhaps a slight increase in drowsiness. Billy was ataxic and drowsy in one or two of the experiments, but usually showed no variation from the normal. In two of the early experiments Blue showed a slight ataxia and hyperactivity, but in the others no effect was observable. The behavior of Lady was more affected by the moderate doses than that of any of the dogs. In two of the seven alcohol experiments (on March 3 and May 4) in her case the ataxia and drowsiness were very marked, so that she swayed on the table and could hardly stand; in some of the others there was occasional unsteadiness.

Effect on conditioned reflexes. Our experiments by the conditioned reflex method gave us information that we were unable to get from simple observations on motor reflexes and general behavior. In Kompa there was no impairment of inhibition, and in fact her inhibi-

tion, which was usually poorly maintained, seemed to be somewhat more stable. The positive conditioned reflexes showed a diminution in intensity, on an average to 64 per cent of the control. There was a retardation of reactivity, shown by the increase in the latent period. This held for both the motor or voluntary muscular system and the secretory systems; the latent period of the former was 135 per cent of the control and of the latter 150 per cent of the control.

The three dogs with conditioned reflexes to a strong and a weak stimulus, showed no loss of differentiation, *i.e.*, no reversal of effect, but there was in all of them a slowing of the reactivity evidenced by the increased latent period: Blue 108 per cent motor and 119 per cent secretory, Billy 118 per cent motor and 123 per cent secretory, Lady 140 per cent motor and 150 per cent secretory. The size of the conditioned reflexes was decreased: in Blue to 80 per cent of the normal for both stimuli; in Billy to 75 per cent of the normal for the weaker of the two conditioned stimuli and 90 per cent for the stronger; in Lady to 83 per cent of the normal for the weak stimulus and to 65 per cent for the strong.

In Kompa and Billy, who were used in the previous experiments, the conditioned reflex is decreased more than with the small dose (see table 2).

There is no constant change in the unconditioned reflex. In Kompa, Blue, and Lady it is decreased (to 91, 90 and 80 per cent, respectively) while in Billy it is slightly increased (113 per cent). But in all the dogs the decrease in the conditioned reflex is 103 to 145 per cent more than in the unconditioned.

3. Large intoxicating doses

Three to 4 cc. per kilogram alcohol, diluted as before to 20 per cent, were given to Kompa, Blue, Billy, No. 3: to Kompa on February 11, 12, 1932; to Blue on March 9, 1932; to Billy on February 25, March 4, December 6, 1932; to No. 3 on November 30, December 2, and 7, 1932.

The behavior was markedly affected and in most cases the animals gave all the signs of being "drunk." Kompa was very wobbly after 20 minutes, did not react to being called, could barely stand, fell on the floor when she jumped up for a biscuit thrown to her. When on the stand, she reeled and rocked and would have fallen had she not been supported by a collar. The same behavior was observed

when she was brought out of the camera after the experiment. The positive conditioned secretory reflexes were considerably reduced in intensity—to 40 per cent of the control. The unconditioned salivary reflex was not diminished—105 per cent of the control, so that the decrease in the conditioned reflex could not be a sign of lessened salivary activity.

The motor system was affected more than the secretory: There was no motor conditioned reflex to the conditioned signals, but the animal turned to the food when it dropped. Thus it is seen that the reflex to the signals involving some delay disappeared before the stronger immediate reflex to the sound of the food dropping, which always occurred at the moment of the feeding; it is known from other experiments that the more nearly a signal coincides with its unconditioned stimulus (food in this case) the stronger it is. However, in the last part of the experiment the animal did not even turn to the food when it dropped; all our artificial motor conditioned reflexes had disappeared. The motor system was more affected than the secretory; for the animal still gave a secretion to the signal even when she had to be fed by hand. The orienting reflex was stronger than usual, *i.e.*, the attention given the signal *per se*, without reference to its quality as a signal. This is an unconditioned reflex, being present in animals from whom the cortex has been removed. It is generally strong at the beginning of the training period, but weakens in most dogs as the stimulus becomes a signal for food. The increase in the orienting reflex here may have been due to the diminution of the conditioned motor reflex to the signal, leaving its other properties (orientation) free. On the other hand, the apparent increase may have resulted from the loss of inhibition which develops in the orienting reflex when a stimulus is often repeated.

There was at one time suggestion of an illusion: the animal turned to the food box when there was no stimulus, an unusual action for Kompa.

Three hours after taking the alcohol, Kompa was still incoördinated; instead of fighting, as she usually did, a pugnacious dog who attacked her, she turned on her back and howled. At the end of 7 hours the incoördination still persisted, but the next day her conditioned salivary reflexes were normal, both as regards excitation and inhibition.

Billy was quite languid and indifferent after large doses of alcohol,

TABLE 1

Dog Kompa. Alcohol experiments. February 12, 1934

ORDER OF STIMULI INTERVAL	CONDITIONED STIMULUS				ORIENTING REFLEX		CONDITIONED REFLEX				REINFORCEMENT (UNCONDITIONED STIMULUS)	SIZE OF UNCONDITIONED REFLEX 60 SECONDS	GENERAL BEHAVIOR: REMARKS
	Time	Conditioned signal	Number of repetitions	Duration in seconds Isolated / Total	Latent period (seconds); strength	Duration (seconds)	Latent period (seconds)		Size of secretion	Motor reflex			
							Secretory	Motor					
1	13:45	B1	359	10–20	+1		1	6	198	+	2	770	
2–5	13:50	M140	393	60	$+^{1-4''}$		—	—	0 0 0 0 0 0	—	—	—	Head on box at 10 seconds
3–6	13:56	Bu	128	10–20	+		1	5	142	+	2	535	
	14:10	50 cc. of 95 per cent alcohol in 150 cc. of H_2O											
4–24	14:20	M60	196	10–20	$+^{1-10''}$		3	—	95	−	2	515	
5–4	14:24	L60	183	10–20	−		4	3	55	+	2	514	Turns to food box at 14:28
6–7	14:31	M140	394	60	+		10		0 13 8 5 } 38 0 12		—	—	Does not put head on box at all after alcohol, wobbly
7–6	14:37	B1	360	10–20			6	—	53	−	2		
	Note in No. 5 food reaction (motor) at 14:28 without stimulus (hallucination)												
	14:38–15:04 Animal definitely "drunk," sways, can hardly stand up; very little reaction to being called												
	Takes food readily, sluggish movements												
8	15:06	B1	361	10–20	$+^{1-6''}$		4	—	61	−	2		
9–4	15:10	M140	395	60	+		—	12	0 0 0 0 0 0	+	—	—	No sleep; does not put head on box
10–4	15:14	Bu	129	10–20	+			—	37	−	2		

					Taken out of chamber until 15:25							
11–14	15:28	M60	197	10–20	+	5	—	13	–	2		
12–5	15:33	L60	184	10–20	+4″	9	—	5	–	2	695	Spontaneous secretion at 15:27
13–4	15:37	M140	396	60	Jerked head into food box —	12	15	0 30 30 25 } 102 5 12	+	—	—	Falling about; hangs head. Head in food box
14–6	15:43	B1	362	10–20	+++		—	70	–	2		
15–5	15:48	M140	397	60	–	<1	—	42 6 22 20 } 100 10 0	–	—	—	Panting, wobbly Panting 30 seconds after M.
16–5	15:53	Bu	130	10–20	<1–8″ ++	4	8	50	+	2	478	Panting
17–4	15:57	M60	198	10–20	+		—	70	–	2		Looks fixedly at M60
18–5	16:02	L60	185	10–20	+3–10″	4	—	19	–	2	548	Ordinarily there is no orienting to L60
19–8	16:10	M140	398	60	+1–8″	18	—	0 36 5 17 } 72 2 12	–	—	—	Hanging head before M140
20–5	16:15	B1	363	10–20	+1–10″	5	—	49	–	2		Does not turn to food, even when shelf drops; has to be fed by hand

When another dog attacked Kompa, she was too incoördinated to fight back, and rolled on her back and howled.

At 23:00 when observed in her paddock she was still incoördinated; could not climb door as usual, did not see biscuit clearly when thrown to her and she stumbled.

and also ataxic; he fell from the table during the experiment, and was also incoördinated afterwards. He did not fight when another dog was brought near him, although he would generally attack any dog in the same room. The conditioned reflexes were irregular and once the weaker one fell to zero, an unprecedented event for this dog. Previously in the experiment he had failed to differentiate between the strong and weak conditioned stimulus, showing reversal of effect, *i.e.*, reacting to the weaker conditioned stimulus in the way he customarily did to the strong one. Billy was a very stable animal and had never showed this reversal before. The next day the differentiation was restored, but the conditioned reflexes continued below normal. The orienting reflex in Billy did not suffer. In the controls (after the reflexes had been well elaborated) this reflex was often absent, and no difference could be noted in the alcohol experiments even with the large doses.

The unconditioned reflexes were reduced to 70 per cent of the normal—to 55 per cent in the case of the weaker and 85 per cent in the stronger.

Blue was ataxic and incoördinated, and fell down while running about the room. This lack of muscular control lasted for 7 hours, after which he was not observed until the next morning. When returned to the cages after experimentation he seemed to lose his fear of the other dogs; he had usually slunk away, cringed and howled, but this time he only ran about the room barking rather aggressively when he was attacked.

In him, as with Billy, there was reversal of effect noticed, so that he reacted to the weak conditioned stimulus more than to the strong. The next day the conditioned reflexes were still greatly diminished or absent, but there was no loss of differentiation between the strong and the weak signals.

The unconditioned salivary reflexes did not show a consistent reduction; for the small amount of food there was a diminution to 75 per cent of normal and for the large amount of food an increase to 108 per cent of normal.

Dog no. 3 did not take the alcohol unless it were diluted to not over 10 per cent. The external behavior showed little or no departure from normal, possibly owing to the dilution. She ran about in circles to the left as she had always done since the removal of the left cortex,

but there was no additional disturbance. In this animal the orienting reflex was always present with alcohol just as it was in the controls; and so was the motor conditioned reflex to the stronger of the two conditioned stimuli. The unconditioned salivary reflexes were reduced to 50 per cent and 87 per cent respectively, for the weak and strong stimuli, and the conditioned reflexes to 0 per cent and 13 per cent, respectively.

SUMMARY OF RESULTS

Table 2 summarizes the results of our experiments, showing the action of alcohol on the conditioned motor and salivary reflexes.

It is important to note that we have paralleled the changes in the conditioned reflexes with those in the unconditioned. The column (no. 10) showing the ratio of reduction represents the comparison between the decrease in the conditioned and the decrease in the unconditioned reflexes. A diminution in the latter would naturally cause the conditioned reflex to fall. For this reason, if we wish to know whether cortical activity is diminished, we must compare the change in the conditioned with the change in the unconditioned rather than accept the absolute value of the conditioned (column 6) as a criterion.

The column "ratio of reduction" shows how the conditioned reflex is changed relatively to the unconditioned, and it is a more accurate measure of the cortical activity than the percentage values in the alcohol-control ratios in the conditioned reflex column. Figures, in column 10, above 100 per cent indicate that the conditioned reflex is decreased more than the unconditioned, and that the alcohol exerts its chief effect on the cortex; below 100 per cent it would mean that the subcortical salivary activity suffers mainly, or that the conditioned reflexes are increased; and when the figures are close to 100 per cent, it shows that there is but little influence on the cortex.

If we follow down the column "ratio of reduction," we find that small doses are almost without effect in normal dogs (average 101 per cent) but that the increase with moderate doses averages 126 per cent, and with large doses 297 per cent of normal (neglecting the pathological animal no. 3 who shows marked changes with each dose).

Chart I brings out the variation of both conditioned and uncondi-

TABLE 2

Effect of alcohol on conditioned and unconditioned reflexes and latent period

DOG	CONDITIONED STIMULUS	NUMBER OF EXPERIMENT-DAYS	CONDITIONED REFLEX			UNCONDITIONED REFLEX			RATIO OF REDUCTION† UNCONDITIONED REFLEX PER CENT / CONDITIONED REFLEX PER CENT	INHIBITION			LATENT PERIOD (MOTOR)			LATENT PERIOD (SECRETORY)		
			Control	Alcohol	ALCOHOL / CONTROL RATIO	Control	Alcohol	ALCOHOL / CONTROL RATIO		Control	Alcohol	RATIO	Control	Alcohol	ALCOHOL / CONTROL RATIO	Control	Alcohol	ALCOHOL / CONTROL RATIO
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
Small doses (0.5 cc. per kilogram)																		
		days	*mm.*	*mm.*	*per cent*	*mm.*	*mm.*	*per cent*	*per cent*	*mm.*	*mm.*	*per cent*	*seconds*	*seconds*	*per cent*	*seconds*	*seconds*	*per cent*
Kompa		15	168	141	85	666	560	84	100	10	10	100	3.9	4.6	118	3.4	3.6	106
No. 3	Weak	6	8	0	0	91	74	81	0	—	—	—	—	2.4	—	13.0	—	+
	Strong	6	10	2.5	25	234	213	91	364	—	—	—	—	3.8	—	6.5	—	+
Billy	Weak	14	343	307	90	375	359	95	105	—	—	—	5.5	6.0	109	4.9	6.4	131
	Strong		523	506	97	814	766	96	98	—	—	—	1.04	1.10	106	3.8	3.4	90

Average large dose (1.5 cc. per kilogram)

Kompa		25	151	106	64	545	504	92	144				4.3	5.8	135		3.2	4.7	150	
Blue	Weak	22	194	156	80	327	272	83	103	—	—	—	3.1	3.1	100	108	3.5	4.0	114	119
	Strong	22	287	226	80	673	673	100	125	—	—	—	2.8	3.3	117		3.3	4.1	124	
Billy	Weak	14	402	303	75	294	321	109	145	—	—	—	7.0	7.5	136	118	2.8	3.1	111	123
	Strong	14	558	492	90	628	731	116	129	—	—	—	2.4	2.4	100		1.7	2.3	135	
Lady	Weak	13	35	29	83	81	62	76	92	—	—	—	2.1	3.2	152	140	17.4	16.4	—	
	Strong	13	127	83	65	173	146	84	129	—	—	—	3.1	4.0	128		8.2	12.3	150	

Large intoxicating dose (3.0 to 4.0 cc. per kilogram)

Kompa		4	143	57	40	537	563	105	262	0	40	0	4.8	11.8	245		2.7	5.0	185	
Blue	Weak	2	165	80	48	280	211	75	156	Differentiation			1.8	7.0	377		2.5	6.0	240	320
	Strong	2	315	50	16	597	646	108	675	impaired for all dogs			3.3	—			2.0	8.0	400	
Billy	Weak	7	355	114	32	280	156	55	172	—	—	—	2.5	5.6	224	144	2.5	6.0	240	223
	Strong	7	456	151	33	672	572	85	260	—	—	—	5.2	3.3	63		1.6	3.3	206	
No. 3	Weak	5	5	0	0	103	52	50	0	—	—	—	—	4.7			6.5	—		
	Strong	5	15	2	13	291	254	87	661	—	—	—	—	—			4.5	5.5	122	

* Conditioned reflex given in millimeters of saliva; 1 mm. of our scale = 0.00167 cc.

† Ratio of reduction (column 10) = ratio $\frac{\text{column 9}}{\text{column 6}}$.

tioned reflexes, and of motor and secretory latent periods dependent on dosage. Chart II shows what we consider the true action of alcohol upon the cortex after correcting for the effect on the uncon-

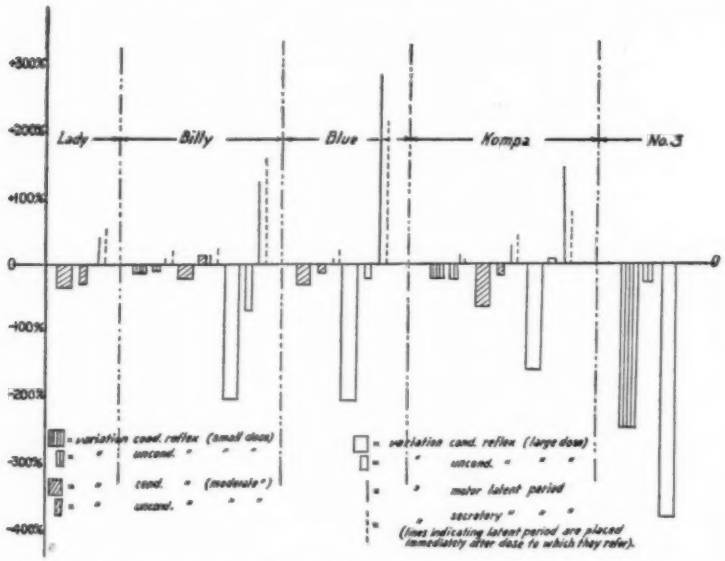

CHART I. EFFECT OF ALCOHOL ON CONDITIONED REFLEXES, UNCONDITIONED REFLEXES, AND LATENT PERIOD IN PER CENT. VARIATION FROM NORMAL CONTROL EXPERIMENTS

○———○ base line = control. Below line = decrease, above line = increase (positive conditioned reflexes or latent period).

(Note: The negative values, below the base line, are calculated by the formula:

$$\text{ratio } \frac{\text{alcohol experiment}}{\text{control experiment}} = \frac{100}{-100}$$

For the actual per cent variations see columns 6 and 9 of the table.)

ditioned salivary secretion. In chart I the control experiments have been made the base line; in chart II the values plotted are the variations of the ratio $\frac{\text{conditioned reflex}}{\text{unconditioned reflex}}$, the ratio in the control experiments having been made the base line.

1. In 41 experiment days on three dogs over a period of two years with doses of 0.5 cc. ethyl alcohol per kilogram body weight we saw no change in the behavior of the animals. The *unconditioned* reflexes (salivary) were reduced slightly—to 84 to 95 per cent of normal. The *conditioned* reflexes were reduced only a little more than the uncon-

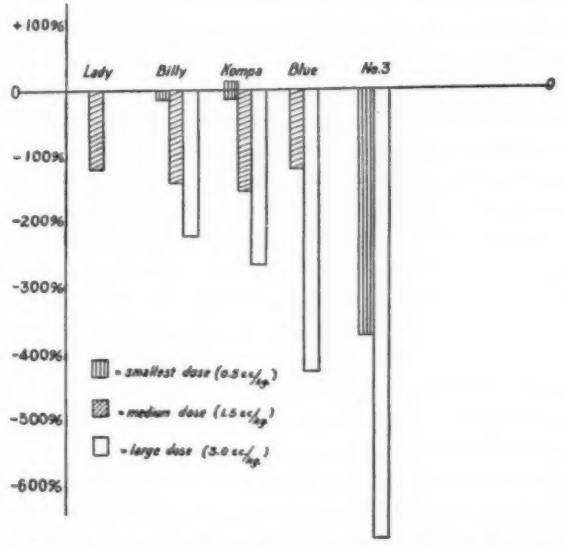

CHART II. EFFECT OF ALCOHOL BY DOSES ON CONDITIONED REFLEXES AFTER CORRECTING FOR ALTERATION OF UNCONDITIONED REFLEXES

Expressed in per cent of variation from normal ratio: $\frac{\text{conditioned secretion}}{\text{unconditioned secretion.}}$

Below line ○———○ = decrease intensity positive conditioned reflex. Above line ○———○ = increase intensity positive conditioned reflex.

ditioned. The *latent period*, both secretory and motor, of the conditioned reflexes was increased slightly, except in one dog with one of the two reflexes, in which the latent period was decreased.

2. With moderately large doses, 1.5 cc. per kilogram, in 123 experiment days on 4 dogs, there was no consistent effect on the *unconditioned* reflex: in 3 dogs a decrease ranging from 80 to 92 per cent of normal, in the fourth dog an increase to 113 per cent. In three of

the animals there was no change in the behavior, while in the fourth (Lady) there was marked ataxia and incoördination in two of the five experiments. In all the dogs the *conditioned* salivary reflexes were decreased more than the unconditioned, from 64 to 83 per cent of normal. The motor and secretory *latent periods* of the conditioned reflexes were increased between 108 and 140 per cent of normal in the motor and between 119 and 150 per cent of normal in the secretory. There was thus a considerable slowing of the reactions in all the dogs. There was no loss of crude discrimination between an inhibitory and excitatory stimulus in one dog, nor between two excitatory stimuli of different intensities in the other three dogs.

3. Using large *intoxicating doses* of 3 to 4 cc. alcohol per kilogram in 32 experiments on four dogs, we found a marked change in the *behavior*—ataxia, inability to stand, drowsiness, loss of aggressiveness. The animals reacted in most cases to being called, and the sleep was always of a light nature. In only a few instances did they refuse to eat, and the food reflex was usually strong, though sometimes they did not react to the food until it was brought by hand close to their mouths. As had occurred with the moderate doses, there was no consistent action on the *unconditioned* salivary reflexes—there was a reduction to 70 per cent of normal in two dogs, in another to 91 per cent, but in the fourth dog an increase to 105 per cent. The unconditioned reflex to the weak unconditioned stimulus was reduced in every case more than the unconditioned reflex to the strong. We have no explanation for this fact; one might think that perhaps the conditioned reflex had an influence on the unconditioned reflex and the removal of this influence in both unconditioned reflexes made the resulting difference larger, as it is known from our previous experiments that there is a much greater difference between the unconditioned reflexes to different amounts of food than is found in the case of conditioned reflexes. However, this view seems untenable in the light of our further experiments showing that the conditioned reflex probably does not continue during the period of the unconditioned, and therefore cannot augment the action of the latter. The *conditioned* salivary reflexes were markedly decreased in all cases (between 6 and 40 per cent of normal in the various dogs). The latent periods of both motor and secretory conditioned reflexes were greatly increased, as much as nearly four times the normal.

Crude *differentiation* between excitation and inhibition was greatly impaired in Kompa, and often lost completely so that she reacted to an inhibitory stimulus as if it were excitatory. The other dogs did not show an inhibitory stimulus, but in them the differentiation between two excitatory stimuli of unequal strength was lost or impaired, so that we often obtained reversal of effect. Frequently, the conditioned reflexes were lost altogether for the 10-second period. The older, natural conditioned reflexes, such as the reaction to the sight and odor of the food, persisted longer than the artificial newer conditioned reflexes which we had formed in the laboratory to the more remote signals to the food; and the immediate conditioned reflex (hearing the food drop down the chute, which always occurs at the instant of feeding) was more stable under alcohol than were the more delayed ones to the signals we employed.

The *orienting reflex*, or the turning of the animal to the conditioned stimulus *per se*, which usually precedes the food conditioned reflex, was not weakened even by the large doses of the alcohol, when the conditioned reflexes were lost altogether. The orienting or investigatory reflex is a strong unconditioned reaction. It is present without training, and also in an animal from whom the cortex has been removed. We are therefore justified in saying that it is a subcortical reflex dependent on the basal ganglia. The fact that it is apparently not weakened may be due to the loss of the inhibiting effect of the cortical conditioned food reflexes. The persistence of a strong orienting reflex after elaboration of the conditioned reflex is an indication of weakened cortical activity or predominance of subcortical excitation, easy distractibility or hyperexcitability. It is usually strongest in the least capable dogs; in the most intelligent animals, as the conditioned reflexes are elaborated, we generally see the orienting reflex, which is at first strong, gradually weaken and finally become entirely replaced by the motor conditioned reflex. The action of the alcohol on the orienting reflex is comparable to that of amytal; in our previous experiments (with H. G. Wolff) with that drug on Kompa this reflex was present long after all the conditioned reflexes had disappeared and the animal was so lethargic that it could no longer take food.

Our smallest dose would not be especially small for a human unaccustomed to alcohol on an empty stomach (three-fourths of a tumbler of port). We can see that, except for the pathological animal,

the dogs "hold their liquor well," as this dose cannot be detected except by a slight increase in the latent period.

The effect on the latent period of the motor and salivary conditioned reflexes is proportional to the dose, and the size (intensity) of the conditioned reflexes is in inverse ratio to the amount of alcohol.

DISCUSSION

The discussion of alcohol recently has concerned itself with the question whether it has a stimulating or depressant effect in small doses. Although it was formerly believed to be a stimulant, Schmiedeberg in 1883 claimed that the influence was due to a removal of central inhibition. In frogs small doses have no effect on the reflexes, but doses of 1 cc. per kilogram in 15 per cent solution (twice our smallest dose) depressed all reflexes (Hyde, Spray and Howat, 2). Rats given 0.65 gram per kilogram, approximately our smallest dose, suffered in both accuracy and promptness in finding their way through mazes (Macht, 3).

Work by the conditioned reflex method in dogs was done by Nikiforovsky in Pavlov's Laboratory in 1910 (Thesis, St. Petersburg) and by another of Pavlov's pupils, L. A. Andreyev (4), at McGill University, simultaneously with ours. After small doses Nikiforovsky saw a removal of inhibition (disinhibition) for the first 15 to 20 minutes, and later inhibition extending to the positive reflexes. Both he and Andreyev found that their dogs became tolerant after several doses, a fact that was observed in only slight degree, if at all, with our animals; *e.g.*, Lady showed the greatest effect in the next to the last experiment. Andreyev used 0.25 cc. of 10 to 20 per cent alcohol per kilogram and found no change in conditioned reflexes, latent period, or simple inhibition.

Andreyev's chief work concerned the production of neuroses by feeding dogs about 4 cc. per kilogram daily for two weeks. At the end of this time he found absolute disinhibition (the complete loss of differentiation between excitatory and inhibitory stimuli), the disappearance of differentiations between positive and negative stimuli that had been stable for twelve months previously, and a lack of adaptability, shown in the fact that to extinguish a positive conditioned reflex required 14 to 16 repetitions instead of the 6 to 7 in the normal. The latent

period was increased during the time that alcohol was given, but when the neurosis developed, it was shorter than normal.

Our large doses, equal to those of Andreyev, were given only two or three times to each dog, and the only after-effect we noted was a disappearance of the conditioned reflexes the next day or a decrease on the third day, but no permanent loss. Our moderately large doses, 1.5 cc. per kilogram, were used ten or twelve times during the month without any impairment on the intervening days.

From numerous physiological and psychological studies it seems fairly well settled that alcohol has a depressant effect on the higher psychical functions. Kraepelin (5) in 1892 was the first to investigate extensively the effect of alcohol on memorizing, adding sums, word associations, etc., and he concluded that the intellectual processes were depressed even with the small doses, but that there was a more rapid discharge of motor impulses, giving way to depression with larger doses. McDougall (6) found diminished accuracy of aim in marking dots on a moving drum with a pin after the ingestion of 30 cc. of whisky, but Rivers (7) did not see any change in the accuracy of typewriting or of multiplication.

Dodge and Benedict (8) have furnished us with an exhaustive report of the effect of 30 and 45 cc. doses of alcohol, given on an empty stomach, to a group of human subjects. They found that the knee-jerk, representing a simple reflex, was depressed—the latent period increased by 10 per cent and the height of contraction decreased 46 per cent. A slightly more complicated reflex, the closure of the eyelid to a sudden sound, had its latent period increased by 7 per cent and the extent of movement diminished by 19 per cent. Reflexes involving higher centers suffered less. Motor coördination was tested by having the subject look rapidly from one point to another and also by tapping the finger. The speed of these decreased 11 and 9 per cent, respectively. Articulation and free associations and memory tests did not reveal any changes. From these studies the authors conclude that alcohol is a true depressant, and that it never has a stimulating action, and furthermore that it influences the lower centers most and the higher centers least.

Later, these studies have been carefully elaborated and, in general, confirmed by Walter R. Miles (1).

Our work is in agreement with Dodge and Benedict's first statement, that alcohol never showed any stimulating effect, and that the depressant action is proportional to the dose. Our experiments, however, would indicate the opposite of what they state in regard to the relative action on the higher and lower nervous centers. As concerns the motor reactions, we have no quantitative measurements except those of the latent period, and this we find increased even with the small dose, a fact that would indicate that the higher functions are quickly influenced. On the other hand, we have accurate quantitative data on the secretory component of both conditioned and unconditioned reflexes. Contrary to the belief of the above authors about the relative action on the higher and lower centers, we found that the *unconditioned* food reflex, as measured by the salivary secretion, was not always decreased even by the largest doses, that the appetite did not suffer, whereas the conditioned food reflex (situated in the higher parts of the nervous system) based on the unconditioned, was markedly decreased. In some of the dogs the unconditioned salivary reflex was even slightly increased by alcohol. This lack of effect on the unconditioned reflex is in agreement with the work of Chittenden (9), who reports that alcohol after ingestion causes no increase in salivary secretion, except for a slight salivation while in the mouth, but Winsor (10) at Cornell found that the unconditioned periodic salivary secretion was decreased in humans. The fact that we did not find any consistent change in the unconditioned is of great importance for the measurement of the conditioned reflexes; for if the former were affected, this would show in the latter.[1] Consistent with this we found that the orienting reflex, *i.e.*, the turning of the animal to the stimulus *per se*, did not suffer except with the large doses. But this is a subcortical reflex depending on the presence of the thalamus and corpus striatum.

Owing to the relatively slight change in the unconditioned salivary reflex, we may consider that our experiments measure accurately the effect of alcohol on both the speed and intensity of conditioned food reflexes in dogs. We had only crude differentiation in our animals, and therefore we are unable to say anything regarding its effect on inhibitions which are difficult or unstable. Inhibition not dependent

[1] We have previously shown in this laboratory that the daily fluctuations of conditioned and unconditioned reflexes are, as a rule, parallel.

on a delicate differentiation was not affected by the small doses. We did not attempt to grade the action according to the time elapsed after the ingestion of alcohol, but took the period of greatest concentration in the blood, *i.e.*, our tests were performed for one to two hours beginning fifteen minutes after oral administration. We did not use any delicate test for the unconditioned motor reflexes, but the changes in the conditioned salivary reflexes appeared long before there were perceptible abnormalities in behavior or motor incoördination.

While Dodge and Benedict draw their conclusions from the measurement of unconditioned reflexes (knee-jerk) and conditioned reflexes (associations, memory) that have no relation one to the other, our experiments record simultaneously the conditioned reflex and the unconditioned reflex upon which it is based. Without knowing the change that occurs in the underlying process, it would seem difficult to analyse and evaluate aberrations in the superstructure. For that reason we think it important to utilize such an activity as the salivary secretion, where we can follow both changes, a correlation that has not been previously made with alcohol.

Our experiments show further that there is never an increase in the intensity of the responses dependent upon cortical connections. Whether this is due to a diminution of the receptor processes, which Dodge and Benedict showed to be a factor in the human, or to a lessening of cortical activity, we are unable to say.

In man, one of the most important effects of alcohol is the emotional; "a man is often kinder but not wiser after alcohol" (Adolf Meyer, 14). Our experiments did not record the concomitant emotional changes, nor do they take into account the varying effect of different alcoholic beverages, nor the associations connected with drinking, change of internal mental scenery, etc. The latter may bring in a whole set of new stimuli. The cases in which alcohol produces beneficial effects may be due to a conservation of energy (1) by diminishing the intensity of the conditioned reflexes and (2) by lessening the tension between excitation and inhibition owing to the failure of differentiation. The lowered threshold of stimulation and the emotional changes, however, might alter the picture and counterbalance these effects by creating a more diffuse and unregulated activity. It has frequently been observed that the use of alcohol does not impair the performance of

those whose work depends upon an active imagination, or emotional setting (littérateurs, musicians, artists), whereas it would seriously affect those whose tasks demand careful deliberations and fine decisions. The chronic effects we do not touch upon; how different these are has been demonstrated by the experiments of Andreyev.

We should like to call attention to the fact that alcohol had least effect on the most stable animals—the two bull dogs—and the greatest effect on the most markedly pathological animal (No. 3). This is especially interesting in the light of previous observations on these dogs in which it appeared that Blue and Kompa were more easily disturbed by unusual shocks in the dog house than the others. Although our series of animals is too small from which to draw definite conclusions, there does seem to be a trend according to temperament or constitution including the pathological. Chart II shows these effects.

In summary, alcohol increases the latent period of both secretory and motor responses even in moderate doses, and in larger amounts decreases the intensity of the conditioned reactions, and disturbs the balance between excitation and inhibition. If and when the behavior of the human can be more clearly resolved into states of excitation and inhibition, and aberrations from the normal can be measured, the experimental knowledge concerning alcohol may become applicable, therapeutically, in cases in which there is an excess of either of these two states.

It is not only a duty, but a pleasure, to express here my great indebtedness to Prof. E. K. Marshall, upon whose advice these experiments were begun, and who has given me valuable suggestions throughout the whole of the work.

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RESEARCHES ON TETANUS

II. The Toxin of the Bacillus tetani Is Not Transported to the Central Nervous System by Any Component of the Peripheral Nerve Trunks[1,2]

JOHN J. ABEL, E. A. EVANS, Jr., B. HAMPIL and F. C. LEE

From the Laboratory for Endocrine Research, the Department of Bacteriology of the School of Hygiene and Public Health, and the Department of Surgery of the Johns Hopkins University

Submitted for publication December 7, 1934

In the years 1884 to 1890, as is well known, bacteriologists definitely established the infective nature of the fatal disease, tetanus, by isolating the causative organism in pure culture. One ordinarily speaks of this organism as the *bacillus tetani*, but it is known to the specialist as *clostridium tetani*, one of the family of the bacillaceae of the general class of plant organisms known as Schizomycetes. That the disease may appear, this clostridium or its spores must be introduced beneath the skin. The history of tetanus shows that there are innumerable ways in which the organism may gain entrance into the body. Thus, it may occur during or after the application of blistering plasters, cupping, extraction of teeth, the application of ligatures to the umbilical cord and to wounds, after slight lesions of any part of the integument, even of the cornea, stings and bites of insects, in the course of gangrene, after scratching acne pustules, after compound fractures and other extensive injuries such as gun-shot and knife wounds, and so

[1] A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of investigations on tetanus.

[2] The senior author has given in an earlier communication a brief account of Professor Lee's experiments on the transection and suture of sciatic nerves into which tetanus toxin was later injected, and also an abstract of our own determinations of the amount of toxin in the blood and lymph of sheep throughout the course of experimentally induced tetanus. The communication appeared in Science, 1934, vol. lxxix, pp. 121–129, and will henceforth be called No. I of our series of papers on tetanus.

forth; in fact, after every kind of lesion of the body surface or of its orifices. At any point of its entrance into the body the bacillus readily multiplies if it meets with suitable conditions for its growth, such as association with relatively harmless pus-forming organisms, as is generally the case, and a diminished tension of oxygen. If washed spores of the bacillus are introduced beneath the skin, their development will take place only if a necrosis of tissue cells is at the same time produced by one method or another. It is well known, too, that the bacilli are not dispersed throughout the body but continue to multiply for a time at the site of inoculation. Only very rarely and under special circumstances are the organisms or their spores found elsewhere in the body. During the time of bacillary growth in the infected area a highly poisonous substance called tetanus toxin appears. This exceedingly potent, water-soluble substance has been definitely proved to be the true cause of the disease. A few bacteriologists believe that the bacillus does not itself produce a toxin but rather a relatively harmless precursor or pro-toxin which is transformed into the true toxin in culture media or at the site of infection in the living animal in the presence of albumose-like substances in the cultures or in the tissues of the infected animal. Nothing whatever is known with certainty of the true chemical nature of the toxin. We cannot even be certain that only one tetanizing poison is produced by the bacilli, but the probabilities are greatly in favor of the supposition that they elaborate a single highly toxic substance. This is being constantly removed from its place of origin by lymphatic vessels and by the blood capillaries, to be then distributed throughout the body by the arterial current. It is evident that we are dealing in the case of this disease with a form of poisoning by a chemical agent. A person with lockjaw, or tetanus, is as truly poisoned as if he had been bitten by a venomous serpent.

According to the generally accepted theory of our day, the disease is defined as being clearly and solely one of the central nervous system; and therefore, in accordance with the prevailing theory, ***none*** of its symptoms can appear until *after* the toxin has reached certain cells of the spinal cord, medulla or pons. Certain of the symptoms of the disease, however, such as the so-called "local tetanus" and the long-continued rigid and unremitting contractures of certain muscles,

lasting for months or even years, that have been observed in human beings and for shorter periods in experimental animals, cannot easily be brought into line with this concise definition of the disease.

LOCAL TETANUS

It is known to every experimenter on tetanus that if one injects a small quantity of the poison into an extremity all of its muscles will, after an interval of time varying from twenty-four hours or so to four days or more, become so rigid that all freedom of movement disappears. The leg is now stiffly extended and so useless that the animal walks on three legs. This condition may exist for weeks without the appearance of a *trace of reflex excitability or any other symptom* of the disease, when only a very small dose of the toxin has been injected. It is to such a condition that the term local tetanus is well applied, but even a single muscle can be thrown into this state of contracture by the injection into it of a very small dose of the toxin. The quantity of toxin that is required to stiffen *all* of the muscles of one hind leg of a dog is less than 1/200 of the usually accepted lethal dose for this animal, as will be shown in a later communication from our laboratory. If the injected dose in the instance cited is very small the local tetanus finally disappears and the animal regains its normal state.

Hundreds of instances of local tetanus involving only certain groups of muscles or even an entire extremity are to be found in the case histories of human beings with tetanus. In these clinical cases, as in animal experiments, the condition may exist for a variable period of time without a trace of reflex excitability. Local tetanus is so very frequently overlooked by the physician, either because he underestimates its theoretical significance, or because by the time the patients reach his hands they already present the more arresting symptoms of the disease, such as difficulty in swallowing, trismus, stiffening of the muscles of the neck and back, or even convulsive seizures of more or less generalized character. Local tetanus must inevitably make its appearance in those muscles of a human being, as in those of a laboratory animal, that happen to lie in the immediate neighborhood of the area in which the multiplying clostridia are producing their toxin.

There is one type of the human disease in which local tetanus is

perhaps never overlooked, and that is the so-called "head tetanus." If the infected area, although it be of limited extent, lies on one side of the face, then only the muscles of that side will respond with local tetanus; in such instances even the trismus may be one-sided. In case the infecting wound involves the top or bridge of the nose, the muscles on both sides of the face will be equally affected. The surgeon Brunner has described, in several very excellent papers, not only the cases of head tetanus that he himself has observed but also a large number of other cases that he has collected from the clinical literature on this type of tetanus. This investigator has also made many striking experiments on animals with toxic filtrates or virulent cultures, in which he was able to repeat and demonstrate every feature of head tetanus as presented by his patients.

MODERN IDEAS ON THE METHOD OF DISTRIBUTION OF TETANUS TOXIN

Long before it was known that tetanus is of infectious nature many clinicians had come to believe that it is truly a disease of the central nervous system. As soon as its true cause had been established, physiologists, pharmacologists, neurologists, pathologists, and students of medicine and surgery began an intensive study of the disease by experimental methods. For them, as for many of the earlier clinicians, its more striking symptoms pointed unerringly to an involvement of certain parts of the spinal cord, medulla, and pons. But that well known symptom called local tetanus presented difficulties in the way of its explanation that could not easily be harmonized with the theory that the disease is solely one of central character. So it came about that the problem that mainly occupied the minds of clinicians and experimentalists forty years ago and has continued to do so down to our day may be formulated in the following words: What single theory will explain both local tetanus and the more generalized or reflex tetanus that is finally superimposed upon the former? Can a mode of carriage of toxin to the central nervous system be proved to exist that will serve to explain not only local tetanus but also that of central origin? Naturally, also the following question stood in closest relationship to the problem as here formulated: Is the toxin transported to the reacting cells of the central nervous system by the blood stream, as is the case with alcohol, numerous alkaloids, and many

other substances, or is there an alternative mode of transportation to the central nervous system for such agents as our bacterial toxin? Neglecting for the moment differences of detail, we may say that a theory was finally evolved which is universally accepted in our day. *The proponers of this theory state most positively that the toxin cannot be taken up from the blood stream by reacting cells of the central nervous system, but that it can reach these cells only when it is carried to them by the peripheral nerves.*

EARLIER EXPERIMENTS IN FAVOR OF THE THEORY OF CARRIAGE OF TOXIN IN THE PERIPHERAL NERVES TO THE CELLS OF THE CENTRAL NERVOUS SYSTEM

The theory that drugs can be carried in peripheral nerves directly to cells of the central nervous system was advanced as long ago as 1867 by the physiologists von Bezold and Hirt, who declared that when the alkaloid veratrine is injected into the sciatic nerve of a frog it passes upward in this nerve with a certain rapidity to reach centers of the spinal cord. Thirty years later, J. Ioteyko concluded from her experiments that, when veratrine is applied directly to the sciatic nerve of a frog, it is rapidly transported downward, in or along the nerve, to a muscle that has been left attached to it. Within two minutes after the application of the alkaloid to the nerve the muscle is thrown into fibrillary contractions, and four to six minutes later a muscle tracing characteristic for veratrine is obtained on faradic stimulation of the poisoned nerve. The author concludes as follows: "Cette action de la vératrine ne peut être expliquée que par le transport du poison le long du nerf; la forme caractéristique de la courbe s'obtient non parce que la vératrine a agi sur le tronc nerveux, mais parce qu'elle s'est propagée le long du nerf et est venue intoxiquer le muscle, en sorte que l'excitation indirecte touche un muscle qui contient de la vératrine."

The experiments of von Bezold and Hirt and Ioteyko with veratrine and the conclusions that were drawn from them were submitted by H. Mies to an experimental and critical analysis in an excellent paper on the blood supply of the sciatic nerve of the frog. It was shown by this investigator that even when either the common iliac or the sciatic artery is ligated at the customary points, as in Claude Bernard's operation, the nerve continues to be well supplied with blood by intrapelvic arteries that enter it above the point at which the main artery was tied. Mies therefore very properly challenged the validity of the conclusions at which von Bezold and Ioteyko had arrived, and pointed out that these authors had failed to take into account the ease and rapidity with which the powerful alkaloid used by them can be carried away from the injected nerve for general distribution throughout the body, even after the sciatic artery had been ligated. Mies, how-

ever, made no reference whatever in his paper to the fact that peripheral nerve trunks are richly supplied with lymphatic capillaries and efferent vessels and that these may be even more efficient than the blood capillaries in removing such substances as tetanus toxin from an injected nerve.

INVESTIGATIONS IN SUPPORT OF THE NERVE TRANSPORT THEORY SINCE 1884 AND THE PROPOSED MODIFICATIONS OF IT

It is impossible to say whether the experiments of von Bezold in 1867 on the supposed carriage of veratrine in peripheral nerves influenced investigators on tetanus nearly thirty years later, and encouraged them to believe that tetanus toxin could be carried in peripheral nerve trunks to the central nervous system. But as long ago as 1884, Carle and Rattone injected into the sciatic nerve of each of four rabbits a suspension of purulent material taken from the infiltrated tissues around an infected acne pustule of a patient who had just died of tetanus, and found that the animals developed the most intense symptoms of tetanus after an incubation period of two or three days. They found, in fact, that such injections of infected material into a nerve trunk were as effective in inducing tetanus as when made directly into the rabbit's dorsal muscles. These investigators were also the first to inoculate rabbits [two] with emulsions of sciatic nerve taken from other rabbits that had been made tetanic by injection of the original tetanogenous material from their patient. Here also: "Il risultato fu uguale; in uno però l'opistotono era meno accentuato."

After it had been fully demonstrated, during the years 1884 to 1890, that tetanus in man and animals is a disease of bacterial origin, by the work of Carle and Rattone in 1884, Nicolaier in 1884, Rosenbach in 1887, Kitasato in 1889, Knud Faber in 1890, and by many others in 1889 and 1890 who corroborated the discoveries of these pioneers, Bruschettini made the important observation in 1892 that, when toxic filtrates of the bacillus tetani are injected underneath the skin of animals, the toxin is found to be present in the nerve trunk in the neighborhood of the injected area. This investigator seems to have been the first to suggest that the toxin of tetanus can be transported from the point of injection in both a centripetal and a peripheral direction in nerve trunks. Meyer, however, calls particular attention to the fact that Bruschettini failed to draw such deductions as could

have been drawn from his experiments in respect to the possibility of their furnishing an adequate explanation for local tetanus, as was done later by him and Ransom. Bruschettini, however, may truly be said to have found the path that has been so well trodden by his successors.

In 1903 Morax and Marie injected the enormous quantity of 3 grams of a powerful tetanus toxin (of which 0.0000005 gram killed mice weighing 15 grams) into the gastrocnemius muscle of the left hind leg of a pony weighing 334 kilograms. The dose injected into this pony, according to our calculations, represented on the most generous estimate not less than 1000 fatal doses for the animal. The pony died of tetanus a little less than three days after inoculation with the toxin. After its death the authors estimated the amount of toxin present in the blood, in a great many nerves of the body, in parts of the central nervous system, in the cerebrospinal fluid, aqueous humor, and elsewhere. The toxicity of these various tissues was determined by injecting extracts of macerated portions of them into mice. The authors found the poison to be present in the blood, in peripheral nerves and parts of the central nervous system, and in the left crural muscle. The sciatic nerve of the left or inoculated leg contained weight for weight more than the corresponding nerve of the opposite side. We cannot consider here in greater detail the analytical reports of Morax and Marie either in reference to their pony or to similar experiments that were made by them on a monkey. Suffice it to say that these authors found that they could demonstrate the presence of the toxin in all classes of nerves, and they therefore concluded "that the three types of peripheral neurons, the motor, the sensory and the sympathetic, are equally able to absorb the tetanus toxin." They finally assumed, however, that the toxin is not carried to the central nervous system either by sensory or autonomic nerves but only by the fibres of motor nerves. It may be remarked in passing that Morax and Marie point out that in a given weight of the sciatic nerve, as for example, when 0.05 gram of the nerve killed mice of the usual weight in two or three days, the amount of toxin contained in this bit of nerve would be exactly what calculation showed should be present in it if the poison were equally distributed throughout the blood and every organ of the body. It is plain, therefore, that even when such enormous quantities of the toxin are injected as in the

experiments of the French authors, no actual concentration of the poison occurs in peripheral nerves.

Meyer and Ransom entered the field at about the same time as Marie and Morax, though Meyer's first paper appeared a year before that of the French authors. Important contributions were made by Loewi and Meyer and Fröhlich and Meyer, and by W. M. Fletcher, who carried on his work in Meyer's laboratory. The investigations of Meyer and his collaborators and the conclusions that were drawn from them have played a leading rôle among the theories of the nature of tetanus that have been proposed during the past thirty years. In all of the papers from Meyer's laboratory the assumption that the tetanus toxin can reach the reacting centers of the central nervous system only by being carried to them in the axis cylinders of motor nerves has been strongly defended by means of well designed and skillfully performed experiments. To many who have not themselves worked in this field this theory of such an unusual and exceptional mode of carriage of a toxin has appeared to be entirely valid and completely irrefutable.

In the hands of Meyer and many later experimenters who gave it their hearty support the theory seemed to give a good explanation of the causation of local tetanus. It has been stated above that this symptom, which may be of long duration, is readily induced in even a single muscle of an extremity when a very minute amount of the toxin is injected into it. The resulting contracture of the muscle is readily explained by the Meyer-Marie theory. The toxin is taken up by the many motor nerve fibres that are dispersed throughout the muscle and is then carried in them directly to motor cells of the anterior horn of the cord. These nerve cells, once the toxin has reached them, now pass into a hyperexcitable state; they become more responsive to sensory stimuli and constantly send out excitatory impulses to the muscles under their control. The appearance of local tetanus is thus fully accounted for; it can occur only *after* the intramuscularly injected toxin (as in our assumed case) has been carried to the central nervous system along this intraneural pathway. This mode of carriage was now generalized to apply to every motor nerve fibre throughout the body. The lymphatics carry the toxin from an infected area into the blood stream, which in turn distributes it to all of the voluntary

striated muscles of the body, and thus serves merely as an agency for supplying toxin to the absorbing and transporting motor nerve fibres of these muscles. The central nervous system cannot take up toxin directly from the blood, but only from the motor axons of peripheral nerve trunks. In other words, the toxin is incapable of passing the "blood-brain barrier." The theory appeared to receive verification from the results of intraneural injections of toxin and of nerve transections both before and after injection of toxin into an extremity. Since the results of such procedures serve equally well to support the other variants of the neural transport theory, we shall consider their evidential value in connection with our discussion of these variants.

Meyer also found strong support for his special theory by reasoning in the following manner. There is but one known continuous path that connects a muscle fibre or an assembled packet of muscle fibres—a so-called motor unit—and the corresponding motor cells of the central nervous system. This pathway is represented by the axons of the motor nerve cells. We have seen that a single muscle can be thrown into local tetanus by injection into it of a minute amount of toxin. Passage of the toxin in these axon pathways meets the requirement of the sharp "one to one correspondence" between a contracture of a motor unit of muscle fibres and the exciting impulses that emanate from its controlling central nerve cells. This very logical way of thinking (the premises being thought sound) induced Meyer to discard an alternative mode of transportation of the toxin, namely, by way of the lymphatics of nerve trunks. He reasoned that if the toxin were conveyed centrally by the lymphatics of a nerve it could not possibly be so precisely apportioned among the limited number of motor nerve cells of the spinal cord that induce local tetanus in a limited number of muscles and only in those that are innervated by the poisoned anterior horn cells.[3]

[3] Goldscheider (Ztschr. f. klin. Med., 1894, xxvi, 175–189) had already stated in 1894 that one is at a loss to explain how the exact localization of the toxin, in the very limited portion of the central nervous system responsible for local tetanus, could be explained by carriage in the lymphatics of peripheral nerves to the subdural and subarachnoid spaces of the central nervous system, and for this reason he refused to accept the nerve transmission theory of toxin. He believed that the

The student of tetanus soon finds that there are wide differences of opinon in regard to almost every feature of the disease. This holds true particularly for the nerve transport theory, as has already been intimated above. There are three variants of this theory: (1) carriage in the nerve fibres themselves—the Meyer-Marie variant which we have been discussing, (2) transport of the toxin from the periphery to the central nervous system and cerebrospinal fluid by endo- and perineural lymphatic vessels of mixed nerves, and (3) centripetal transport in the tissue spaces or *Lymphräume* of nerve trunks.

Let us examine each of these assumed variants of the nerve transport theory more carefully.

1. Further Remarks on the Theory of Carriage in Motor Nerve Fibres

We have already made some observations of a critical nature concerning this variant of the general theory but more must be added. Meyer and his associates state very definitely that this mode of carriage can proceed only very slowly and that the toxin is transported centripetally as well as centrifugally in axis cylinders by a process that is termed "protoplasmic streaming." It may be pointed out that there is no experimental evidence whatever that there exists anything in the nature of a slow "protoplasmic streaming" or current that can carry the molecules of the toxin from point to point in axis cylinders, as is the case when it is injected into a vein or a lymphatic vessel with its moving current. Secondly, when the toxin is found to be present in an excised piece of nerve, such as the sciatic, in the neighborhood of the point of the injection, there is no trustworthy evidence that an appreciable amount of it *is present in the axis cylinders themselves* and that the blood capillaries and the endo- and perineural lymphatic vessels of the nerve do not contain practically all of it. It

primary local tetanus and the later more generalized tetanic convulsions are both to be explained by a central action of the toxin, and he also assumed that the toxin could so alter the state of excitability of axon terminals that their corresponding neurons in the cord would in some unexplained manner be themselves rendered more excitable by this action of the toxin on their peripheral terminations in the muscles.

is well known that the lymphatic capillaries readily absorb the toxin from any injected area and carry it into the blood stream, and there is no sound reason for believing that those of a nerve trunk do not also absorb it if given the opportunity. Too much has been made by the proponents of this variant of the nerve transport theory (as well as by those who champion its other variants) of the well known fact that the part of a nerve nearest the point of injection can be shown to contain some of the toxin. Sawamura, an ardent supporter of the neural transport theory, admits that no part of the sciatic nerve can be shown to have taken up the toxin unless at least two fatal doses of it have been injected subcutaneously into the leg, and that it is always found only in that part of the sciatic nerve that lies nearest to the point of injection of the toxin. Both Permin, also an ardent supporter of the nerve fibre transport theory, and Pochhammer state that after the injection of toxin into the nerve of a leg its presence cannot be detected in the upper part of the sciatic nerve unless large quantities of it have been injected. Permin succeeded in finding it only in the upper part of the sciatic of a large guinea-pig (experiment of July 6, 1911, page 22 of his paper) after injecting into the calf of its left leg so large a quantity of toxin as to kill the animal in twenty-four hours! He is much puzzled to explain the absence of the toxin in the upper part of the nerve after the injection of several fatal doses into the leg, and suggests that this is due to the rapid upward carriage of the toxin. He forgets that in accordance with his own observations intraneurally injected toxin rapidly escapes from the nerve into the surrounding tissues and muscles and that after a time the nerve itself contains no more toxin than the tissues immediately surrounding it. The fact that the more centrally-lying parts of the sciatic are found to contain no toxin unless heroic doses have been injected into a leg is now readily explainable. The many efferent lymphatic vessels of the upper portions of a nerve trunk constantly remove whatever part of the toxin has been taken up by the lower portion of the nerve. This fact cannot be reconciled with the theory of the carriage of toxin to the spinal cord in the neural lymphatics.

Pochhammer, who has propounded the singular theory that local tetanus is brought about by an action of the toxin in destroying the myelin sheath of nerves, both motor and sensory, and that these

bared nerves now permit the direct passage of a sensory impulse *from a bare sensory nerve* to a similarly bare neighboring motor nerve, thus producing contracture of muscles by a process of short-circuiting of impulses, makes the following claim on the basis of his experiments. He states positively that local tetanus appears in the muscles of the hind leg of a rabbit that has received a subcutaneous injection of the toxin *before* the toxin can be detected in the sciatic nerve itself. It is quite impossible to bring this and other observations mentioned above into line with the theory of a steady upward movement of the toxin in nerve fibres.

It has been stated that Meyer believes that the carriage of toxin in nerve fibres proceeds very slowly. In his opinion and in that of his later defenders this very slowness of transport affords a good explanation for the well known period of incubation that always intervenes between the injection of toxin and the appearance of the first symptoms of tetanus thereafter. Singularly enough, however, other competent investigators disagree sharply with Meyer. The surgeon Permin describes in detail certain experiments on rabbits that have led him to believe that the transport of toxin in nerve fibres proceeds not slowly but with great rapidity ("ausserordentlich rasch"). According to him it requires only four to six hours in the rabbit for the toxin to pass from the distal part of its sciatic nerve to its spinal cord. Sawamura, who has worked very intensively at tetanus, came to much the same conclusion as Permin but some years earlier. He concluded from experiments much like those performed by Permin that the conduction [of the toxin] in peripheral nerves proceeds "pretty rapidly" in them, according to his observations. ("Die Fortleitung in peripherischen Nerven geht nach meinen Untersuchungen ziemlich schnell.")

Quite aside from this difference of opinion among investigators as to the rate of carriage of the toxin in nerve fibres, it is generally believed even in our day that the period of incubation is much longer in the larger animals, such as the horse and man, in which it may vary from three days or even less to twenty or more days, than is the case in very small animals. In the mouse and guinea-pig, after experimental inoculation, the period may be as short as from eight to twelve hours; in the rabbit it is set down as varying from eighteen to thirty-six

hours. Treatises on tetanus give tables of varying periods of incubation for animals from the size of the mouse to that of the horse, such as the one drawn up by Courmont and Doyon. In these tables the period of incubation is always given as one of hours in the small animals, and then as increasing progressively until it reaches three to five days or more in the larger animals. Data of this kind were thought by Meyer and others to support their theory, as it is quite evident that the toxin would require much more time to ascend the long sciatic nerve of a horse, for instance, than the much shorter sciatic of a guinea-pig. But the belief that the period of incubation is always of short duration in the very small animals, as compared with the large ones, does not rest on a firm foundation. It has been found in our laboratory, for example, that a properly selected subcutaneous dose of toxin will give for the guinea-pig the same incubation period of three or four days as a similarly selected dose will give for so large an animal as a sheep weighing three hundred times as much as the guinea-pig. Many factors must be taken into consideration in making positive statements concerning the length of the incubation period in the case of any animal species. Similar factors come into play in causing the extremely variable periods of incubation as observed in the natural disease in man and in the equidae. The amount of toxin produced at the site of infection, or of that inoculated into an experimental animal, its virulence, the locus of infection or inoculation, whether this be in, close to, or at a distance from muscles, the amount of daily labor that is done by a human being or the amount of running about that is done by an animal after infection or inoculation and many other factors, all have a marked influence on the length of the period of incubation.

One of the most important determining factors is undoubtedly the amount of toxin that is injected at any one point of the body. This was well shown by Morax and Marie in their experiments on a pony, referred to above. It will be recalled that a very large dose of a powerful toxin, amounting to 1000 fatal doses or more, was injected into the gastrocnemius muscle of a pony's left leg. On the morning of the following day local tetanus had already made its appearance in the injected leg, and lockjaw and general tetanic convulsions followed rapidly. We have here, then, an example of the great shortening of the incubation period after *intramuscular* injection of a very large dose of the toxin to twenty-four hours or less. But it is after the *intravenous* injection of large doses that the shortest

periods of incubation are found. Thus Marie, who pointed out as long ago as 1897 that the period of incubation "is essentially variable and depends in particular on the quantity and activity of the tetanic poison," found that a rabbit that had received intravenously half a milligram of a very active and standardized toxin died of generalized tetanus on the fourth day, while a second rabbit on receiving 100 mgm. of the same toxin, also intravenously, died of tetanus in twelve hours. Our own experiments with guinea-pigs show that these animals will succumb to generalized tetanus within eight to ten hours following direct injections into the heart of 8,000 to 20,000 lethal doses. After the injection of such large amounts the animals are apparently quite unharmed and show no noticeable manifestations of tetanus until seven hours have elapsed. Then there appear quite suddenly the extreme symptoms characteristic of the disease, and these persist until death occurs two or three hours later. Two control animals injected in the same way, one with an equal quantity of the uninoculated medium and the other with a toxin that had been boiled to destroy its potency, showed no ill effects during an observation period of two weeks.

It was stated by Marie that the minimal intravenously injected lethal dose of the toxin for a rabbit is eight to nine times larger than that required to kill one of equal weight into which the toxin is injected under the skin; while Knorr found that the intravenous minimal lethal dose is twice that of the subcutaneous. Quadu asserts that in guinea-pigs the intravenous lethal dose is six times larger than the subcutaneous. Fildes found that the relation for the rabbit is as five to one when the intravenous mode of injection is compared with that into the muscles. This supposed striking difference between the lethality of intravenous and other modes of injecting moderate amounts of the toxin is surprising and quite out of line with the results of similar injections of many poisons, such as alkaloids, glucosides and other pharmacological agents.

A year ago van den Hoven van Genderen for the first time in the history of tetanus gave serious attention to this matter and by the use of a sufficiently large number of animals obtained data that carry conviction to the reader's mind. He finds that in the case of rabbits and guinea-pigs, quite contrary to the belief of earlier investigators, no material difference can be established in the time of death following intravenous, intramuscular, or subcutaneous injections of from one to three or four lethal doses of toxin, expressed in terms of guinea-pig minimal lethal doses. We ourselves had already arrived at the same conclusion in experiments on animals other than those studied by van den Hoven van Genderen though our evidence was based on a smaller number of cases. Our experiments with sheep and with dogs have shown that when one injects from one to four or five lethal doses, while there may be slight differences in the time of the first appearance of symptoms, the time of death is practically independent of the mode of injection. We have purposely omitted here all reference to intracerebral or intraspinal injections of the toxin. Their significance can be discussed more profitably in a later paper in which local tetanus with and without reflex or central nervous involvement will be described.

The data given above seem to prove that the shortest periods of incubation in all animals thus far tested attend the intravenous injection of very large doses of the toxin, such, however, as would probably never come into play in the natural disease. An interesting feature of the physiological action of the toxin is seen in the fact that even when very large doses of the poison are injected intravenously no alteration of blood pressure, respiration or the general condition of the animal is discernible until many hours have elapsed. Courmont and Doyon tested this matter on a dog by injecting as much as 258 cc. of tetanus toxin (143 lethal doses) into the jugular vein at a rate of 1 cc. every 18 seconds, and found no changes in blood pressure, cardiac rhythm or respiration, during the three hours the animal was on the operating table.[4] After detaching the manometric apparatus the animal was found to be quite "normal" and two hours later (9.00 P.M.), when the last observations of the day were made, it presented no noticeable symptoms of poisoning. On the following morning a slight incoördination of gait had appeared. At 1.15 P.M. the forelegs and the neck were stiff; a few hours later the animal died in general convulsions. Here again in this experiment of Courmont and Doyon we find that intravenous injection of an excessive amount of toxin is followed by an incubation period of hours, and that death inevitably occurs shortly after the first appearance of rigidity in the extremities.

We believe, then, that as in the natural disease in man, so also in experimental work on animals, one cannot assign a definite lower and a definite upper limit to the period of incubation, and that variations in this period are readily accounted for by taking into consideration the factors just enumerated. Nevertheless, one fact stands out prominently both in the natural disease and in experimental work with animals, namely, that under ordinary conditions in which only from one to two fatal doses of toxin come into play a number of days supervene between natural infection or subcutaneous inoculation and

[4] Meyer ("Tetanus Studien," in Jaffe's Festschrift, p. 306) also measured the blood pressure of a number of dogs in advanced stages of tetanus and found that it differs practically not at all from the normal blood pressure of dogs. In tetanus he found that it varied from 130 to 140 mm. Hg. During the convulsions it rose to about 140 mm., sank thereafter for a short time to about 120 mm. and then rapidly regained its original level of 130 to 140 mm.

the first symptoms of the disease. In other words, the poison with which we are here concerned manifests its action *slowly* in all species of animals that are susceptible to its action except under quite exceptional circumstances, as when truly enormous doses of it are injected intravenously. We have pointed out that one can make the period of incubation in any species of animal either short or long, and that tables that attempt to set definite limits for the period have little significance from a scientific point of view and none whatever in the way of lending support to the nerve fibre transport theory. We have marshalled much evidence against the validity of this theory, but many more facts that cannot be brought into harmony with it or the two other variants of the neural transport theory will be presented incidentally in later papers dealing at greater length with local tetanus and other features of the disease.

2. *The Toxin Is Supposed to Be Carried to the Central Nervous System by Way of the Endoneural and Perineural Lymphatic Vessels*

This second variant of the nerve transport theory had its early proponents in Gumprecht, Stintzing, Cyrus Field, and others, and was strongly supported by the investigations of the pathologist Aschoff and his collaborators Reinhold and Robertson that were made during the world war. Teale and Embleton, whose investigations also fell in that period, likewise accept this second or lymphatic mode of transport in addition to carriage by nerve fibres. Horster and Whitman have made many experiments as recently as 1931 to show the possibility of this mode of carriage of the toxin, and their work has been cited with approval in this very year by Friedemann and Elkeles as supplying corroborative evidence "for the conception that the toxin reaches the spinal cord and the brain exclusively by way of the peripheral nerves." It is evident that this postulated mode of conveyance of the toxin to the submeningeal spaces in the closed channels of the lymphatics cannot be regarded as one that proceeds very slowly, as was assumed to be the case by Meyer and his school for their theory. Once the toxin has been taken up by the lymphatics of the peripheral portions of a nerve trunk it must proceed centripetally at the rate of flow prevailing in these vessels. This second variant of the neural transport theory, in contradistinction to the first, offers no explanation

for the long period of incubation[5] that so often precedes the appearance of local tetanus in an extremity that has been injected with the toxin. Were this theory correct, one could certainly expect a quicker central response to the action of the toxin with a more rapid appearance of convulsive seizures. Investigators that defend this second, like the defenders of the first variant of the theory, rely to a very great extent on injections of toxin into nerve trunks for their evidence, and they appear to be firmly convinced that all neural lymphatics have a free entrance into the cerebrospinal fluid and the substance of the cord. Their ideas are based in large measure on the monumental investigations made in the early 70's of the last century by Key and Retzius. As is well known, these investigators injected gelatin solutions containing Berlin blue into the subarachnoid cavity of human cadavers and found that the blue salt found its way along peripheral spinal nerves to a considerable distance from the spinal cord. They concluded that definite pathways exist along which the cerebrospinal fluid can easily pass into the perineural spaces and even into the very substance of peripheral nerves. It may not be out of place to cite a passage in which they describe the distribution in peripheral nerves of the material that had been injected into the subarachnoid cavity. "Ins innere der Nervenbündel setzt sich ferner oft die Flüssigkeit von den perineuralen Spaltenräumen mehr oder weniger zwischen den an gewissen Stellen sich einbiegenden endoneuralen Lamellen in die endoneuralen Spaltenräume fort. Sie breitet sich dabei in grösserer oder geringerer Ausdehnung in den inneren Theilen des Bündels aus, indem sie erstens hauptsächlich zwischen den sich immer weiter vertheilenden Lamellen verläuft und die Nervenfasergruppen in dieser Weise umspült. Die injicirte Flüssigkeit bleibt aber nicht in den endoneuralen Spaltenräumen, sondern breitet sich auch hie und da von denselben in die Nervenfasergruppen selbst aus. Ihr Verhalten zu letzteren ist etwas eigenthümlich. Sie bleibt nicht in den Spalten ausserhalb der Fibrillenscheiden, noch dringt sie nur in diese hinein, sondern sie füllt den ganzen Raum ausserhalb und innerhalb der Fibrillenscheiden, bis zu den Schwannschen Scheiden."

The anatomists of our day have become very skeptical of the con-

[5] We shall offer our own theory in explanation of the period of incubation in a later paper.

clusions that were drawn by Key and Retzius from their experiments. They believe rather that the high injection pressures employed by them on cadavers (about 60 mm. Hg.) account for the escape of their Berlin blue solution in the subarachnoid cavity far out along the spinal nerves. Robert Elman, working in the laboratory of Professor Lewis H. Weed[6] of the Johns Hopkins University, has made careful histological studies of the region of the spinal cord where the spinal nerve roots emerge, after subarachnoid injections of isotonic solutions of a non-toxic substance, and has not been able to demonstrate "the existence of a fluid pathway physiologically connecting the subarachnoid cavity with the so-called perineural space."

Iwanow and Romodanowsky corroborated Elman's findings a few years later as a result of their own studies "on the anatomical connection of the cerebral and spinal submeningeal spaces with the lymphatic system," without, however, being acquainted with Elman's work. After injecting especially prepared suspensions of India ink and certain non-diffusible colloids into the subarachnoid spaces of both living and dead animals, these investigators found that the injected substances were not carried away from the subarachnoid cavity by the spinal nerve roots. Microscopic examination of the roots showed that they had taken up only a certain amount of the India ink but this was never carried farther in them than the intervertebral ganglion. So far these authors are in agreement with Elman, but they now go on to say that their observations support the well known belief of Speransky's school that "fluids travel in nerves (*Nervenscheiden*) toward the brain and not in the reverse direction." Elman gives no hint whatever in his paper that he subscribes to this mode of transfer of fluids in peripheral nerves.

We cannot here do more than allude in passing to the main objective of Iwanow's own researches and those made in collaboration with Romodanowsky. These authors believe that they have realized their objective and have discovered that a not inconsiderable part of the cerebrospinal fluid escapes from the subarachnoid space in lymphatic

[6] Professor Weed has made some sound critical remarks on the observations of Key and Retzius in relation to the drainage of the cerebrospinal fluid, in his "Studies on Cerebrospinal Fluid," published in the Journal of Medical Research, Boston, 1914, n.s. xxvi, no. 1, p. 45.

vessels of a special type that have their origin in the dura mater and is carried in them to many of the abdominal and thoracic lymph glands and to the intestinal mucosa and Peyer's patches. Since it is assumed by these writers that this flow of cerebrospinal fluid in special lymphatics is centripetal and unidirectional only, we need not concern ourselves further with it and may leave it for anatomists to decide whether this mode of drainage of cerebrospinal fluid actually occurs under normal circumstances. Acquiring our information from others we had assumed that a lymphatic system has its origin in the lymph spaces of the cerebral layer of the dura mater; that it drains into the superior deeply-lying cervical lymph nodes; that "*this system is closed, however, on the cerebral surface of the dural membrane and has no communication with the pia-arachnoid spaces.*"[7] (Turner and Reynolds, p. 12.)

Implications of the Theory

The theory of a free flow to the central nervous system in the neural lymphatics now under consideration implies, in the first place, that all the lymph of peripheral nerves mingles ultimately with the cerebrospinal fluid, and secondly, that any substance whatever, be it a useful drug or a toxin, that finds its way into peripheral nerves and is devoid of destructive action on them must finally reach the central nervous system. In recent years Speransky and his pupils Wischnewsky and Ponomarew have gone so far as to make the following statements in regard to these matters. Thus Speransky writes, "Il s'ensuit donc que les liquides du tronc nerveux montent, quoique lentement, mais sans interruption, et avec une vitesse variable, dans la direction du cerveau et s'insinuent partiellement dans le segment correspondant. Ils emportent dans leur courant des substances de composition chimique très variée, qui y ont pénétré d'une facon quelconque." And his pupil Wischnewsky states that[8] "the lymph of a nerve trunk flows in a steady slow stream in the direction toward the brain and carries with it all substances that have entered it, provided these substances are indifferent in their action on local tissues and do not destroy their structure. Not only tetanus and

[7] Italics ours.

[8] Translated from the German.

diphtheria toxin but also many other substances which, in this or that manner, have entered a nerve trunk are carried forward passively in the nerve trunk. The forward movement (*Fortbewegung*) of the lymph in a nerve trunk, like the movement of other fluids in the body, depends upon the difference of pressure at the beginning and end of the way. By raising or lowering this pressure at the beginning or end of the route (*Bahn*) the rapidity of transfer of the fluid in a nerve can be voluntarily altered." In all of these statements the end point of this flowing current in peripheral nerves is always found in the central nervous system. Many experiments have been made by Speransky's pupils, Ponomarew and Wischnewsky, in support of these surprising statements. They have injected dye stuffs and tetanus toxin into nerve trunks and have attempted to note changes in their rate of passage after altering the pressure of the cerebrospinal fluid either by withdrawing various amounts of it or by an ingenious operative procedure, in which a cuff of muscle tissue is placed around the spinal cord itself in such a manner that the upper part of the spinal subarachnoid cavity is completely cut off from its lower division. All of these experiments, it will be noted, and the deductions that are drawn from them are based on the underlying assumption of a free passage of fluid from nerve trunks to the subarachnoid spaces. We shall not consider at this point in further detail the experimental findings of these authors, since their assumption will now be shown to be baseless.

It is surprising that none of the proponents of the theory of carriage of toxins and other drugs by way of the neural lymphatics have ever asked themselves the simple question whether their assumption of a physiologically occurring centripetal flow of lymph in nerve trunks has any foundation in fact. They seem never to have inquired whether the anatomists of our day have studied this matter and whether they have discovered at what points the lymph of nerve trunks leaves them and where it goes. In 1930 Aldo Defrise, in numerous neural injection experiments on newborn babies and on dogs, studied the mode of origin of the lymphatic capillary networks of peripheral nerves, and came to the conclusion that this network is composed of a closed system of lymphatic capillaries which are not open to the interlamellar spaces of the perineurium. He studied the epineural efferent lymphatic "collectors" of the median, crural, sciatic,

peroneal (communis), and posterior tibial nerves, tracing these lymphatic vessels to the lymphoglandular groups into which they empty. We cannot here enter into further details of this anatomist's work and the precautions that he has taken in regard to the materials used in his injections, pressures, and other points of technique. Jossifow and his associates Shdanow and Kurdümow have also published very extensive researches in this field since 1930. Shdanow has traced the course of the lymphatics of the nerve trunks of both the fore and hind limbs of man. The efferent lymphatics of the nerves of the hind legs, for example, take their course along the arteries of the nerve and throw their contents into more deeply-lying "collectors," but in all cases the lymph finally passes through regional lymph nodes onward into the thoracic duct. Nowhere in the writings of the anatomists here cited is there even a suggestion that any neural lymphatic vessels enter the spinal canal at any intervertebral junction. The lymphatics, for example, at the upper part of the sciatic reach deeply-lying inguinal glands and those of its lumbo-sacral trunk finally empty into the hypogastric lymph glands. In a word, we derive no evidence from these recent studies of anatomists that the lymphatic vessels of nerve trunks pour their contents into the submeningeal spaces of the central nervous system, and it would seem to be quite impossible longer to maintain that tetanus toxin or any other substance that may have been taken up by the neural lymphatics can be carried in them to the central nervous system.

We may remark in passing that we are leaving out of consideration all reference to the anatomical conditions that exist in the olfactory and optic nerves, as neither of these can properly be classed with the peripheral nerves.

In concluding this section of our paper a few remarks will be in order on the work of Horster and Whitman, alluded to above. These investigators, like their very numerous predecessors (none of whose experiments can be described here), have studied the effects of injecting the sciatic nerves of rabbits with trypanblue and with tetanus toxin and have found that these substances can be driven very rapidly into the cerebrospinal fluid. Thus, in one of their many experiments with trypanblue, in which 0.05 cc. of a strong solution of this dye was injected into the right sciatic nerve of a rabbit at a point 4 cm.

below the spinal cord, it was found that the spinal cord was stained blue for a distance of 2 cm. above the cauda equina. In this case the animal was killed immediately after the completion of the injection and the spinal cord was at once examined. In another experiment (rabbit 15) 0.08 cc. of the trypanblue solution was injected into the right tibial nerve at a distance of 2 cm. above its entrance into the muscle, and in so short a period as five minutes after the injection clonic seizures appeared in both hind limbs. Similar experiments were made with injections of tetanus toxin. One sciatic nerve in each of a series of twelve sizable rabbits was exposed under morphine-ether anesthesia and a concentrated solution of tetanus toxin was injected into the nerve trunk. The volume injected varied from 0.04 to 0.1 cc. These amounts of toxin represented from 4,800 to 12,000 lethal mouse doses. At various periods following the intraneural injections cerebrospinal fluid was removed by occipital puncture and was tested for its content of toxin by subcutaneous injection into white mice. In five out of the twelve experiments the authors found that the spinal liquor was toxic for mice in quantities varying from 0.25 to 0.65 cc. In these successful experiments, as in experiments with trypanblue, the toxin appeared *very soon* in the cisternal liquor. When the cisternal liquor was found to contain no toxin within two to three hours after the intraneural injection, it was never found to be toxic when tested at any time in the following 24 to 48 hours. In a word, when Horster and Whitman did not succeed in *driving* the toxin into the lumbar cerebrospinal fluid at once by syringe pressure, the cisternal fluid never contained any of it later, even when the authors waited almost three days for signs of its appearance. No more conclusive evidence could be offered than that given by Horster and Whitman themselves that the high injection pressure of a syringe can drive dye stuffs and toxin very rapidly into the submeningeal spaces of the lumbar spinal cord. Furthermore, an examination of their unsuccessful experiments, that is to say, those in which they failed to drive the toxin at once into the submeningeal cavity, leads us to a very important conclusion. In all of these unsuccessful experiments the solution was propelled only a certain distance up and down the nerve and not far enough centrally to reach the submeningeal spaces, *and yet in all of the experiments of this nature, although the toxin was dis-*

tributed throughout a certain length of the nerve trunk, it ceased to travel further along the nerve as soon as the pressure of the injection had fallen to a low level. In a word, even when there is a good deal of toxin or dye stuff present in a nerve trunk *it cannot be carried into the central nervous system by forces naturally inherent in the nerve or by its lymphatics.* The authors themselves remark that under these circumstances the toxin "could not reach the submeningeal spaces by a secondary centripetal transport from the intraneural depot." . . . "dass grössere Farbstoff- oder Toxinmengen zwar durch die intraneurale Injektion als solche in den Liquor bzw. in die submeningealen Räume geschafft werden können, nicht aber durch sekundären zentripetalen Transport von einem intraneuralen Depot aus."

3. On the Carriage of the Toxin in the Tissue Spaces of Nerve Trunks

Peripheral nerve trunks, like other tissues and organs of the body that are richly supplied with blood and lymphatic vessels, contain tissue fluid that fills the so-called tissue spaces. Anatomists hold that no true lymphatic capillaries are found in the bundles of nerve fibres that compose a peripheral nerve[9] but tissue spaces, or "*Lymphräume*," exist around every nerve fibre and between the thin layers of the perineural sheath surrounding bundles of such fibres. These minute spaces with their numerous interposed barriers are supposed to continue on in the nerves through intervertebral structures and foramina right into the subarachnoidal and subdural spaces of the spinal cord and brain. They do not join up in any way with lymph vessels that may be present in the epineural tissues. The amount of tissue fluid that is supposed to be present in these minute spaces must be very small indeed in the normal and uninflamed nerve, since peripheral nerve trunks are classed with the less watery structures of the body. There is not the slightest evidence that the metabolites present in the tissue spaces or toxins that have found their way into them can be transported from one point to a more distant one by any but the slow process of diffusion. However, in recent years Speransky and his co-workers Wischnewsky and Ponomarew have asserted, indeed, if we read their papers correctly, that tetanus toxin can be carried to the central nervous system in these neural tissue spaces. It has already

[9] Shdanow: Anat. Anzeiger, 1931, 71, 231–245.

been stated that these investigators accept the theory of carriage of toxin by the lymphatic vessels of nerve trunks, but to this theory they have added the mode of carriage that we are now considering. To quote a paragraph from a summary given by Wischnewsky in one of his papers, "The various chemical agents introduced into the peripheral part of a nerve spread out in the nerve path (Nervenbahn) in a centripetal direction; these substances can not only reach the central part of a nerve but obviously also the spinal cord itself. *The paths by which they spread are the perineural lymph or tissue spaces*[10] (Lymphräume)." The term Lymphräume is invariably used by anatomists writing in the German language as the equivalent of the English term *tissue spaces*. We have then no other recourse than to believe that the Speransky school assumes, in part at least, centripetal carriage of toxins in these minute spaces.

We have seen that the second variant of the nerve transport theory is entirely without foundation, now that it is known that the lymphatic vessels carry the lymph of nerve trunks into lymph glands and then on to the thoracic duct, and we believe that the theory that the toxin is conveyed to the central nervous system by way of the tissue spaces of nerve trunks is equally untenable. The tissue pressure at peripheral points is not of sufficient magnitude to move solutions through such narrow spaces, frequently interrupted as they are by barriers. These minute spaces are energized by molecular forces only (surface energy and diffusion) and it remains to be proved that such forces can compass the astonishing feat of transporting a poison for long distances, as in the sciatic nerve of a horse, and through intervening cell barriers to the central nervous system, in any period of time that could ever come into consideration either under experimental conditions or in the naturally occurring disease, tetanus. Diffusion can be effective only in a short space of time when it operates in structures of minute dimensions, as in pulmonary alveolar capillaries and other absorbing cell layers whose thickness is only about a thousandth of a millimeter.

We have already emphasized the well known fact that there is only one method known to physiological science by which a substance can be *rapidly* transported from one point to another of an organ, and that

[10] Italics ours.

is by way of the blood or lymphatic circulation. It has also been pointed out that tetanus toxin or a dye stuff, such as trypanblue, can be introduced into a peripheral nerve trunk under low pressure and that, although under these conditions the tissue space mechanism of the nerve is given every opportunity to operate, it is nevertheless entirely unable to carry anything into the subarachnoid spaces of the central nervous system. What part of the injected substances is present in these tissue spaces of the nerve is soon carried away from them by blood and lymphatic capillaries.

We need not spend more time in discussing this question of the assumed, more or less rapid movement of solutions in the tissue spaces of long peripheral nerves.

DISPROOF OF AN EARLIER ATTEMPT TO SHOW THAT WATER SOLUBLE SUBSTANCES CAN BE DISTRIBUTED THROUGHOUT THE BODY BY THE "TISSUE SPACE MECHANISM"

It is a curious fact that this theory appears to intrigue investigators from time to time, and it may be well to comment briefly on the work of S. J. Meltzer, who thought he had proved the existence of a perfect mechanism for the distribution of substances throughout the tissue spaces of the entire body. In 1911 this investigator, in commenting on his numerous experiments with alkaloids and acid fuchsin on frogs said "My experiments demonstrate that in the absence of central circulation substances may be distributed through the body by a mechanism which in some instances may act even more promptly then the cardiac mechanism. In contradistinction to the central apparatus we may designate the distributory agent in question as the peripheral mechanism. The path of distribution employed in this mechanism can be nothing else than the tissue spaces." Meltzer's work was done on cardiectomized frogs, which were shown to react, in the entire absence of the circulation, with great rapidity toward very small quantities of acid fuchsin, strychnine and morphine. In fact it was found that the effects were much greater and appeared more rapidly than when the drugs were administered to normal frogs.

As the senior author of this paper and his collaborators had been working on the action of convulsive dye stuffs on frogs before Meltzer

published his theory and were engaged in their investigations at the time of its appearance, they could not do otherwise than put his theory to the test of experiment. It was found that Meltzer had failed to take into account the rôle that was played in his experiments on cardiectomized frogs by their still actively beating lymph hearts, in the carriage of his drugs from the injected area to the central nervous system. When the four lymph hearts of a cardiectomized frog are completely destroyed or excised no effects whatever follow the injection of any centrally acting dye stuffs or alkaloids into a subcutaneous lymph sac. It was furthermore shown that Meltzer's supposed peripheral mechanism was utterly incapable of distributing any substance whatever *throughout the entire body of a cardiectomized frog, when all of its lymph hearts were intact.* In fact, the intact lymph hearts of Meltzer's cardiectomized frogs were able to transfer substances that had been injected at any point beneath the skin *to one organ only, namely, the spinal cord and brain.* It was found that, when the heart is tied off, the beating lymph hearts can take over only a part of the distributory rôle formerly played by the cardiac mechanism; they can carry drugs with an almost equal rapidity to the central nervous system, but only into this division of the frog's body. The explanation was found in the fact that although the lymph hearts can no longer push their lymph into the heart, since this organ has been tied off, they can still send it directly into the system of veins that drain the central nervous system. In this manner, acid fuchsin, strychnine and the like, that have been taken up by lymph hearts, are carried directly in these veins to the meninges of the brain and spinal cord, and this *reverse current* is made possible by the fact that these veins are entirely valveless.

The reader will find many more data in the experiments that were carried out by the writer and his associates in the years 1910 to 1914 that will convince him of the utter ineffectiveness of "the peripheral mechanism" as a distributing agent for chemical principles in the absence of the cardiac and lymphatic circulations. The reader will also find further experiments in support of our belief that a water-soluble substance, not in itself destructive of tissues, that has been injected at any point in the body of a frog cannot under normal conditions reach the cells of the central nervous system, completely

closed as it is in its bony encasement and well guarded by connective tissue elements at all intravertebral junctions and foramina, except by the usual route, namely, that of an intact cardiac circulation.

SUMMARY AND ADDENDA

We have presented many considerations and many facts in support of our belief that tetanus toxin and dye stuffs injected in an aqueous medium either intraneurally, subcutaneously, intramuscularly or intravenously are not carried in the axis cylinders, the lymphatic vessels, or the tissue spaces of peripheral motor nerves to the reacting cells of the central nervous system. We have also cited the recent investigations of anatomists who have traced the outflow of lymph from nerve trunks and have shown that it, like the lymph of other structures of the body, is added finally to the venous blood and not to the cerebrospinal fluid.

We furthermore called attention to a series of investigations that were carried out by Abel and Abel & Turner in the years 1910 to 1914, in which it was conclusively shown that alkaloids and dye stuffs cannot be distributed throughout the body by any peripheral mechanism, such as the "tissue spaces."

An account will be given in later papers of experiments that have been in progress in our laboratories for more than two years on the pathogeny of local tetanus, on the influence of complete denervation of muscles on the course of the poisoning, and on the reflex phenomena and other aspects of both experimental and natural tetanus. We find ourselves quite as unable to accept the current theories in regard to many of these characteristics as we are to accept the nerve transport theory, for the very good reason that this untenable theory is here also made to serve as the basis for their explanation.

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BOOK REVIEW

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

Atlas Fundus Oculi. By DR. WILLIAM HOLLAND WILMER. Introduction by Dr. Warfield T. Longcope. 100 color plates. $35.00. (*The MacMillan Co., New York, 1934.*)

Dr. Wilmer has completed his brief but momentous career at the Johns Hopkins Medical School with the publication of an atlas of ophthalmoscopy. He has been engaged in the preparation of this volume for the major portion of his stay in Baltimore and the work, therefore, may be counted as an extremely condensed synopsis of the clinical experience at the institute during this period and also as an illustration of the meticulously detailed clinical analyses that were done under the routine which he established. The book will furnish important aid in the teaching of ophthalmology both in this country and abroad for a generation to come.

Ophthalmoscopic diagnosis is such an essential part of ophthalmic practice that it can never be taught too thoroughly. Often it is difficult to be sure that the teacher and student are both seeing the same thing, when examining the eye of a patient. For the beginner, at least, the demonstration of fundus drawings is therefore essential. The need for such aid was recognized soon after Helmholtz had invented the ophthalmoscope in 1851, and in 1853 von Tright, in a monograph on the instrument, published a number of black and white drawings and one color plate illustrating the appearance of the eyegrounds in health and disease. Numerous similar brief compilations followed, but the first serious attempts at the production of a colored atlas of the eyeground were made by Liebreich in 1863 and by Jaeger in 1869. Numerous lesser works followed, but until the close of the century, Liebreich's and Jaeger's were the standard works.

At the end of this period the need for a more modern and better atlas became felt and three different atlases were published almost simultaneously—Haab, 1895, Frost, 1896, and Oeller, 1896–1904—to be followed by Adam in 1912. Special mention should also be made of the work of Loring, who produced in 1886–1891 the only atlas of ophthalmoscopy published in this country before that of Dr. Wilmer. It may be interesting to note that Loring also spent at least some part of the period of preparation of his work in Baltimore.

These five collections of pictures, together with some lesser works have served as the breast milk for infant ophthalmologists until today. All of them are vastly superior in the quality of their drawings to those that had appeared before, but only that of Oeller is free from such gross simplifications in the execution of the paintings and in lithographic reproduction as not to be misleading to the be-

ginner. Oeller's pictures are superb both in detail of drawing and in accuracy of reproduction.

These atlases of a generation ago, useful though they have been, suffer from several disadvantages. Many of the conditions that are well known to the profession today were unrecognized at the time these older atlases were made, and many details in the ophthalmoscopic lesions of commoner ailments are better understood now than they were a generation ago. The advance of medicine has been such in the last few decades that the clinical data and descriptions accompanying these older drawings often seem totally inadequate, and the demonstrator frequently has to supply an hypothetical clinical history to go with the picture.

These defects have been remedied by the publication of Dr. Wilmer's atlas. With each of the hundred cases presented, a full clinical history is given, together with detailed reports of a careful general physical examination, and the results of all the modern routine laboratory tests. The fundus drawings by Mrs. Burgess are, in the opinion of the reviewer, superior to any that have been published heretofore.

In view of the general excellence of the book it may seem unfair to carp at details, but the work of the lithographer is not as good as that of the author and the artist, nor is it quite as good as that in Oeller's atlas. It would appear that the lithographer has used a magenta red as one of his fundamental colors which has given an opaque quality and sometimes a slight purplish tinge to some of the plates, thus missing the luminous warm quality of the color of the eyeground which is so well reproduced in the artist's original paintings. It is often said that American lithographers can not do quite so well as those abroad, but the profession which has produced such magnificent examples as, for instance, the Audubon prints, or to come nearer home, the anatomical and embryological illustrations published under the direction of Dr. Mall—the profession which has produced these masterpieces has no excuse for not equalling or even surpassing the work of foreign lithographers.

Dr. Wilmer's chapter on the technique of ophthalmoscopy furnishes many useful hints to the beginner, and Dr. Longcope in the introduction has given a valuable summary of the field of usefulness of ophthalmoscopic diagnosis in relation to internal medicine. The publishers are to be complimented on the general format of the book. Finally, the profession owes a debt of gratitude to the members of the Wilmer Foundation who through their financial support have made possible the publication of this book at a price that is within the horizon of the individual student.

J. S. F.

3

THE DIAGNOSIS OF DISEASES OF THE LUNGS WITH SPECIAL REFERENCE TO TUBERCULOSIS[1]

CHARLES R. AUSTRIAN, M.D.

Let us turn back for the moment to the time of Stuyvesant or Schuyler and come for a first visit to the state of New York. Assume that we are skilled woodsmen, versed in all that is known of the trees of the temperate zone and are on a quest to identify the arboreal flora of the district. At first, it will be difficult to see the trees for the woods. The primary task of identifying a vegetative growth as a tree will be simple and many varieties will be differentiable more or less readily from others. Next, trees that seemed at first to be very different will be found to belong to the same family. The separation of evergreen from deciduous types will be relatively easy. The identification of species of pines, firs, spruces, poplars, oaks, maples and others will be more difficult, and in isolated instances accurate classification of individual growths may require prolonged research.

Move now through the two centuries that have passed since those pioneer days and approach the same problem with the knowledge acquired in those years. Initial identification of most families and genera will proceed rapidly and with certainty, fewer growths will remain difficult to classify and many differentiations unnoted before will be made with relative ease. Nor will this latter-day survey be initiated from the same primary base nor with methods altogether new. Facts acquired by previous investigators will be reviewed and revalued, tried methods will be utilized and supplemented or complemented by newer ways of study.

The exploration of the forests of pulmonary diseases parallels in every way that of the primeval and of the cleared woods of this and of other terrains. Approached in the days just after Auenbrugger, Corvisart and Laennec 'twas relatively simple to recognize among diseases of the lower air passages certain large groups of maladies—acute and chronic, benign and malign, bronchial, pulmonary and

[1] Friday Afternoon Lecture, New York Academy of Medicine, November 24, 1933.

pleural, primary and secondary. But with the cruder ways of study, many groupings that were made proved to be too inclusive and some, such as the differentiation of tuberculosis and phthisis, too refined. Sally forth for a new survey of respiratory diseases today and as in the modern effort to classify the sylvan growth, the task will be a different one. The investigator will or should be informed of what is known already and be adept not only in newer methods of study, but skilled in the utilization of those tested means that had advanced knowledge before. So, in trekking through the mazes of the forest of broncho-pulmonary diseases, the facts established by earlier explorers will be kept in mind, the proved methods of physical diagnosis will guide to the establishment of major classifications, and modern procedures will discover more precise groupings. The old that is good will be respected in spite of its age. The new that is of doubtful or negative value will be rejected although it is novel.

The establishment of the clinical significance of a disease of the lower respiratory tract is a complex problem that may be resolved into several essential components. First, the determination of the structural defect. Secondly, whether or not the morphological change is accompanied by impairment of local function. Thirdly, to what extent such functional derangement is associated with systemic disturbances, and finally, what is the specific cause of the morbid process.

Generally, structural alterations of the lower airways can be detected by the ordinary methods of physical diagnosis (inspection, palpation, percussion and auscultation) supplemented by roentgenography and fluoroscopy and at times by bronchoscopy. The value of these procedures is determined by the skill with which they are employed and by skill is meant more than technique. Acute senses and practice in the use of diagnostic methods give technical facility, but the data gathered will be of diagnostic value only insofar as they are correlated with anatomical conditions and with functional activities. Such correlations will be accurate only if they are founded upon a knowledge of normal as well as of pathological anatomy and physiology. Since the time of Laennec, physicians have sought to perfect themselves in the art of physical diagnosis and it was chiefly by the use of the four fundamental procedures employed ordinarily that most pathological changes of the lower air passages came to be known

clinically. Of these methods, no more need be said than is required to emphasize a few salient points. Often, if a disease of the respiratory tract is suspected, the helpfulness of inspection is lessened because the extrathoracic manifestations of the condition are not looked for (cyanosis or pallor, type and rate of respiration, anisocoria, clubbing of the fingers, etc.) and the viewing of the thorax itself is cursory or not recorded. Frequently, likewise, palpation is either quite neglected or is carried out perfunctorily and without regard to the information it may give. The helpfulness of percussion to detect alterations of density within the chest is limited, not merely because it is an art difficult to master, but often because the examiner fails honestly to estimate what information he can derive from the use of it. Most observers will agree that of the usual methods of physical examination inspection and auscultation are to them the most reliable and informative in the detection of an antomical abnormality of the lower respiratory tract. As practiced ordinarily, these procedures are often insufficient and even when employed skillfully fail to reveal many foci of pleural, bronchial and pulmonary disease. Because many physicians can not become adept in the use of these methods and because of their admitted inadequacy always to discover foci of pleural or pulmonary change, accessory procedures have found a ready welcome. Of these, the X-ray stands preeminent.

Not long after Roentgen and others discovered how the X-ray could be employed to disclose pathological changes within the chest, it came into use as a means of thoracic exploration. Many regarded the procedure as a shorthand way to demonstrate the presence, site, size, clinical significance, even the etiology of diseases of the respiratory tract, and used it as a substitute for, rather than as a supplement to, other diagnostic investigations. It serves to check the accuracy of a physical examination, to locate, outline and determine the extent of foci of disease, to disclose pathological changes undetected by other means because of their location or because of associated physical conditions, to localize some foreign bodies and to give a permanent graphic record so that the advance or retrogression of a pathological process may be followed accurately. The roentgen-ray has a rôle of prime importance in the diagnosis of thoracic disease but utilized as it is by some, it is at times a source of misinformation. The use of it should

be limited to those trained in anatomy, physiology, pathology and clinical medicine as well as in technique. The worker should know the normal appearances in the various decades of life to be able to recognize the abnormal, and should realize that, although no thoracic examination can be considered complete without a roentgenograph of the chest, the latter can not take the place of a careful history and physical examination. It is patent that although the film may show evidences of anatomical changes, it can not discover the clinical disturbances of disease nor determine with certainty the etiological factor concerned. In every instance, the data furnished by roentgenographic study must be checked with those derived from the clinical history, physical examination and the studies of the laboratory. In short, the X-ray is helpful:

(1) As a check upon the informative worth of ordinary physical examination. It demonstrates that minimum local signs correlated with suggestive symptoms can not be accepted always as final evidence of structural changes and that the data furnished by inspection and auscultation are associated with altered roentgenographic appearances more regularly than are those gathered by means of palpation and percussion. Further, when the ray indicates the nidus of disease is smaller than physical exploration indicates it to be, atelectasis is present and often, when the contrary is true, emphysema or hyperinflation of the lung causes the inaccurate estimation of the extent of the change.

(2) In many cases, it discloses pathological changes not discoverable by other methods of examination such as pulmonary lesions that are far distant from the parietes, without bronchial communication or contact, that are covered by the scapula, that lie within an interlobar fissure, in the posterior mediastinum or in the trough of the diaphragm, or that are masked by a thoracic deformity, by a pleural exudate, a pneumothorax or by emphysema. Thus, so-called silent cavities, primary or metastatic tumours or foci of suppuration, areas of beginning pneumonia or infarction, localized pneumothorax or small pleural effusions or tumours may be discovered first when a radiograph is made; or a diagnosis of diffuse seeding of the lungs with abscesses, tubercles or tumours may await confirmation until a film of the chest is available.

But the X-ray, too, is fallible. It is well known that certain types of foreign bodies are not demonstrable by means of it, that fibrinous pleural exudates fail often to cast diagnostic shadows and, in rare instances, pneumonic consolidation may not be revealed in films of the chest.

An ordinary roentgenograph of the chest may not disclose the fact that bronchi are dilated and the introduction into the bronchial tree of a radio opaque substance may be needed to show that bronchiectasis exists.

The complete diagnostic utility of the X-ray will not be available, however, unless it is cast laterally and tangentially as well as in the postero-anterior or reverse direction. These projections have made it possible to recognize that shadows due apparently to abnormalities of the pulmonary parenchyma were really attributable to fluid encapsulated in the pleura. Finally, if stereoscopic roentgenography has revealed the presence of an abnormal density in the lung or pleura, it may localize the pathological change with greater accuracy if employed again after the induction of pneumothorax.

Thoracentesis is useful not merely to prove the presence of fluid in the thorax and to determine its nature, but to disclose the presence, location and size of a pleural or pulmonary lesion that was masked by it. Aspiration alone or aspiration followed by the introduction of air into the chest is at times a valuable adjuvant to physical and to roentgenographic examination.

The internist looked at the thorax, percussed and listened to it; he X-rayed the chest, visualized its contents and found that still many pathological conditions eluded detection. Therefore, he welcomed the advent of bronchoscopy. This method has been especially helpful in determining whether or not there is obstruction to or dilatation of the trachea or larger bronchi. Like other special methods, it is a supplementary one and its value is limited. Positive findings are of great value; negative ones are usually of little importance. By means of bronchoscopic inspection, the presence and the nature of an obstruction of the tracheo-bronchial tree may be established; the cause of haemoptysis or of dyspnea, of atelectasis or of some forms of asthma may be determined and the diagnosis of pulmonary or of bronchial suppuration be verified and the site of it localized. Moreover, endo-

bronchial examination may be the means to determine the etiology of broncho-pulmonary disease, as it affords a way to obtain uncontaminated secretion or tissue for bacteriological and microscopic examination. The chief contraindications to examination with the bronchoscope are advanced cardiovascular disease, aneurysm of the aortic arch and haemoptysis that is not due to a foreign body or to an endobronchial tumour.

This history and the general examination of the individual tell of the disturbances of function for which an anatomical defect may be responsible. They give the criteria on which the clinical significance of the structural fault and on which our ideas of the pathogenesis of the physical disease are to be based. The helpfulness of the information derivable from these sources will vary in diseases of different etiology; but the facts contributed by them are the indispensable, final criteria of the clinical significance of morbid processes. The importance of intrathoracic abnormalities will be stressed or minimized insofar as they can be related causally to derangements of function, local or remote.

The specific cause of a pulmonary disease may be suggested by the symptoms of the patient, by the localization and character of the pathological changes, by alterations of the constituents of the blood, by serological reactions, by allergic responses and by other special tests, but ultimately it is established by the demonstration of a specific biological, chemical or physical noxious agent.

The symptoms of the many diseases of the lungs are legion and none is pathognomonic. It is the relative frequency of some in certain maladies and the absence of some in others that gives to each its peculiar significance. No one disease alone affects a given section of the pulmonary field but tuberculosis elects the upper third of the lungs and non-tuberculous infections involve more often the inferior portions of them; changes due to mycotic infections, to pneumonoconiosis, to metastatic abscesses or neoplasms and to generalized tuberculosis are usually bilateral; those due to primary tumours of the bronchi or lungs, to foreign bodies, to hydatid disease, or to solitary abscess, are generally unilateral.

Hematological changes are informative only to a limited extent. Anaemia of severe grade is not the rule in phthisis but develops more

frequently if there is malignant or extensive suppurative disease of the air passages. Eosinophilia speaks for a mycosis, polymorphonuclear leucocytosis for a pyogenic infection and lymphocytosis or mononuclear for tuberculosis. The alteration of the monocytic lymphocytic ratio described by Dr. Sabin, the Arneth shift of the leucocytic formula and the increased sedimentation rate of the red blood corpuscles are collateral evidences of value in determining the activity of phthisis.

Serological reactions are of limited utility. The complement fixation test has not proved of practical value in the clinical study of tuberculosis but it has in determining the presence of hydatid disease. The demonstration of specific agglutinins in the blood may prove a broncho-pneumonia to be due to bacterium tularense. Even if the therapeutic response is prompt, a positive Wassermann reaction should not be considered proof that a focus of pulmonary disease is syphilitic unless other causes are lacking or the treponema pallidum can be demonstrated in the sputum. In our judgment the casual association of a positive test and a pulmonary lesion is taken too often to be a causal one.

The demonstration of specific hypersensitiveness is helpful in the study of bronchial asthma, and of the tests for sensitiveness to bacterial substances those made with tuberculin, with streptothricin and with bacterium tularense are the most informative. The several tuberculin tests have been utilized extensively to determine the presence of infection with the tubercle bacillus. Although a positive reaction does not necessarily indicate the existence of clinical disease, considered in conjunction with certain physical or roentgenographic signs, it has come to be the basis on which the incidence of tuberculous infection and of so-called latent tuberculosis has been determined. More sensitive than the Pirquet test, the intradermal test with tuberculin is the final qualitative and quantitative index of sensitiveness to Koch's bacillus, provided it is made properly. Just as the demonstration of tubercle bacilli is the one way that of itself alone proves positively a diagnosis of tuberculosis, so the tuberculin test is the only way that unassisted can establish the absence of that infection provided the few conditions can be excluded that are known to abolish the local response to ordinary amounts of tuberculin.

A positive reaction to any one tuberculin test determines only the

presence of infection with the tubercle bacillus—the intensity of it does not indicate whether or not the infection is active or significant clinically, but in the main a vigorous reaction is indicative of a recent infection or a recent extension of an old one.

Speaking generally, the specific cause of a pulmonary disease is demonstrable more often by examination of the sputum than by any other single investigation. Therefore it is essential that the examiner know what constitutes sputum—the source of what he is to study, how it is collected and how it is to be treated. The material selected may be examined fresh, by means of dark-field illumination, stained with various dyes, and culturally. Repeated studies are necessary oftentimes to confirm a positive finding and to fortify the implication of a negative one. It is true generally that if numerous proper examinations of purulent sputum fail to show the presence of a suspected pathogene, a different etiological factor should be sought for.

Let the truth of the preceding statements be stressed in the discussion of pulmonary tuberculosis that follows.

The ubiquity of pulmonary tuberculosis, in every stratum of society, at all ages and in all communities has led to an intensive study of the disease. During the first decade of this century in particular, efforts were made so to enlighten physicians that a diagnosis could be made when the disease was in what was termed the "incipient stage." The minimal evidences of phthisis were made known and emphasis was laid on the ways to identify small foci of disease, but strangely enough, in this era, comparatively little stress was put on the symptoms of active infection, and it was some time before the relative significance of the systemic manifestations and of the local signs of the disease was established. The more recent investigations have coördinated clinical histories, tests with tuberculin and physical and roentgenographic surveys. Such studies have elaborated and altered accepted ideas of the incidence and pathogenesis of tuberculosis and have made it possible to recognize forms of tuberculosis not discoverable by other means.

To define what is meant by incipient tuberculosis is difficult. The term should be used to designate the earliest detectible stage of the disease. It can not signify "incipient" in a chronological sense for an infection may remain symptomless and without signs for many

months or even years. Nor can it connote "incipient" in the sense that the pathological change is a minute one, for often small foci of disease are or have been associated with severe or incapacitating symptoms, and extensive areas of tuberculous changes may exist without local or somatic disturbances. The ability to recognize the disease promptly just before or when it begins to have clinical significance will depend on a knowledge of the pathogenesis and pathology of pulmonary tuberculosis and upon familiarity with the disturbances of function that it may cause. The changes of structure give rise to local signs and may be responsible for focal symptoms. The alterations of the general metabolism give rise to systemic manifestations. The presence of local signs determines the anatomical activity of the process, the occurrence of symptoms establishes its clinical significance. To differentiate between anatomical and clinical tuberculosis is of practical importance and the procedures to establish this differentiation are well recognized. None is more important than a carefully and intelligently assembled record of the patient. The family history is useful to establish whether or not there has been intimate domiciliary contact with tuberculosis, not because it is believed that heredity plays a significant rôle in the transmission of infection, but because prolonged contact with the tubercle bacillus is recognized as the vital factor in its dissemination. Until comparatively recently, the liability to contamination in childhood was stressed and the importance of infection in adult life was estimated lightly. More recent studies have indicated, however, that the menace of Koch's bacillus to the adult is a very real one.

The personal history of the individual may be most informative, for though the symptomatology of pulmonary tuberculosis is varied indeed, the cardinal manifestations are comparatively few. Among these, of prime significance are haemoptysis, pleurisy with effusion, fever, rapid pulse, loss of weight and lack of endurance. Chills, sweats, thoracic pain, cough, expectoration and digestive or menstrual disturbances, though important, are less significant.

Imbalance of the thermo-regulatory mechanism, a wide spread between the daily maximum and minimum temperature even if the former is only slightly above the normal line, prolonged, unexplained fever, persistently rapid or very labile pulse rate, diminished capacity

to endure without discomfort effort that formerly was not productive of tiredness, frequent respiratory infections, ill-defined disturbances of digestion, fistula in ano, inanition without anemia, amenorrhea without signs of pelvic or of endocrine disease and not a concomitant or a sequel to a systemic malady are symptoms that demand, in addition to a general survey, particular investigations to determine the presence or absence of pulmonary tuberculosis.

Valuable markers of the diagnostic highway that any or all of the foregoing symptoms may be, their directional import will be lost if it is not realized that they point often to other conclusions. None is pathognomonic, none is more than presumptive and the definiteness of the presumption each affords is enhanced or lessened by its association with local signs of thoracic disease. These signs are determined by a complete physical survey and are established finally as indicative of a tuberculous or a non-tuberculous disease by special investigation.

The local physical signs due to the usual type of pulmonary tuberculosis may be foretold if the pathologic anatomy of the condition is kept in mind. Both clinical and pathological studies have established the tendency of the so-called adult type of the disease to involve predominantly the upper part of the lungs. An apical or infra-apical localization of abnormal signs engenders at once the thought of tuberculosis. Such anatomical alterations, even if slight, may cause delayed or lessened respiratory excursion of the upper part of one side of the chest and this is more significant if it is associated with a slight flattening of the hemi-thorax that is not caused by a skeletal abnormality. So long as the structural alteration is not marked, the transmission of vocal fremitus will not be changed appreciably and there will only be a slight diminution of resonance. Observers disagree concerning the value of percussion to discover slight modifications of density, for no absolute standards of what is a normal note can be set up. The size, shape, thickness and resilience of the thorax, the volume and elasticity of its contents and the posture of the patient are variables encountered in different individuals. The auditory and tactile acuity of examiners, their appreciation of pitch and quality of sounds are not the same, so that it can not be stated categorically what changes of the note are indicative of intrathoracic disease and what

are not. Each examiner must orient himself and know how much he can rely upon the evidence he gathers. Suffice it to say that relatively slight change of density in the summit of a lung may be revealed to the skilled observer by a diminution of resonance in the supra- or infra-clavicular region, over the supraspinous fossa or, even more readily, by a narrowing of the width of apical resonance. These changes will be more informative if they are associated with such auscultatory signs as a similarly localized diminished or increased transmission of the breath sounds, a wavy type of inspiration and prolongation of expiration or an elevation of the pitch of the respiratory murmur. The latter are frequently the earliest objective evidences of pulmonary infiltration and may be overlooked readily.

The most constant and significant sign of tuberculosis of the lungs is a shower of medium moist inspiratory râles heard constantly over one or the other apex, in the first or second interspace, or above the spine of the scapula with or after expiratory cough. It is well to note that auscultation over the body of the scapula discloses abnormalities now and again and that the apex of the axilla should be explored in every examination. In patients with recent infiltration, these râles are not audible during quiet breathing, but are heard only during forced respiration or are elicited by a quiet cough made at or near the end of expiration. They are audible with the effort of coughing or during the early part of the succeeding inspiration. No type of râle is pathognomonic of tuberculosis. Persistence and site are more significant than the character of the adventitious sounds. Over deeply situated foci, râles may be audible even when the resonance is not modified. Diminished resonance and a changed respiratory murmur may be the only physical signs of an inactive process and dry râles or crackles may persist long after a focus has become quiescent or healed. Transitory râles are of little significance.

The roentgenographic changes in this stage of phthisis are well recognized. If the disease is active anatomically, the film will show single or multiple, minute or small ill-defined shadows along the broncho-vascular radiations in the infra-apical or apical areas, denser at the center, more nebulous toward the peripheral margins which are hazy and ill-defined. There may be a coalescence of such shadows with lighter areas between them indicating that the infiltration of the

pulmonary tissue is not complete. Such shadows are due to conglomerate tubercles and to the exudative reaction of the pulmonary tissue. The evolution of them indicates well the progress of the disease. Increased peripheral haziness, the first appearance from time to time of similar areas, coalescence of some of them, clearing of others, the development of shadows due to cavities or to pleural exudation —any or all of these mutations may persist or accompany the development or increase of local and constitutional symptoms. Conversely, decreasing haziness and better definition of individual shadows with or without evidences of calcification, clearing of clouded areas and the replacement of accentuated, fuzzy, thickened lines along the bronchovascular trunks by sharply demarcated threads and lacings—due to fibrosis—may occur with, precede or follow symptomatic improvement of the patient. Bands of increased density along the interlobar fissures are seen frequently. Diffuse, uniform haziness indicative of thickened pleura and increased shadows in the hilar regions are usual, too.

There are many individuals with the cardinal symptoms of tuberculosis in whom the most skillful physical examination discloses no sign of pulmonary disease but whose thoracic roentgenograph pictures slight or even extensive pathological changes. Because of this fact roentgenography is an absolutely essential part of the study of individuals thought to have any disease of the lungs. Conversely, because now and again the roentgenographic film shows no anatomic fault even when tubercle bacilli have been demonstrated in the sputum, the X-ray can not be estimated as the sole and final criterion to exclude a pulmonary lesion.

Several conditions that may simulate closely this so-called adult type of phthisis merit comment, and diagnostic pitfalls gape widely if the examiner is driven by hurry and is satisfied by presumption instead of by proof. The way to avoid them is to shun haste and not to cease the study of a problem until it has been solved.

Because systemic disturbances may be in the foreground and local symptoms and signs may be inconclusive or wanting, the thoracic origin of an illness may be unsuspected. In that not unusual event, the erroneous conclusion drawn with embarrassing frequency is that the pathological state is due to some extrapulmonary disorder.

Apical or subapical râles may be associated with an infection of the paranasal sinuses. In such cases other evidences of pulmonary infiltration, physical and roentgenographic, are lacking and the adventitious sounds disappear when the local condition has been corrected.

Occasionally a subsiding edema of the lungs or an incompletely resolved pneumonia of an upper lobe may be responsible for signs like those enumerated, and this is true especially of the bronchopneumonia that occurs as a complication of influenza. Since the pandemic of 1918–19, many mistaken diagnoses were made because this fact was not realized. Frequently, the history, the blood picture, repeated study of the sputum, and the evolution of the pathological changes are needed correctly to establish the nature of the illness.

Partial atelectasis limited to the upper third of one or both lungs due to the narrowing of a bronchus, by tumour, inflammation or foreign body or rarely as the residuum of a massive collapse of the lung may also cause similar signs. Such conditions are to be suspected if the mediastinal structures are drawn to the affected side in the absence of fibrosis there, in which event a bronchoscopic examination is indicated.

As the tuberculous involvement of the lungs spreads, it may give rise to an almost incredible variety of symptoms and signs and be responsible for syndromes acute or chronic. If the diversity of the pathological changes and of the consequent functional disturbances in phthisis are borne in mind, the protean nature of the disease will be understandable; if it is realized that none of these effects is decisively characteristic, the diagnostic problem will be better appreciated.

Acute types of pulmonary tuberculosis may at a given time be indistinguishable from acute pneumonia, lobar or broncho, of other genesis. However, one clinical difference is significant often before other informative evidence is available. Speaking generally, whether the inflammatory process involves the superior or inferior portion of the lung, whether the febrile reaction, cyanosis, polypnea and tachycardia be moderate or severe, if the pneumonia is tuberculous the patient seems less severely ill than if it is due to a pyogenic organism. In fact, if an individual with the signs of pneumonia is able to be ambulatory or appears to be only slightly ill, there is a clinical presumption that his local changes are due to the tubercle bacillus. Lapse

of time and the evolution of the local process, or the demonstration of the specific cause of the syndrome, may be needed to determine its etiology.

The diagnosis of what is styled the juvenile type of tuberculosis may be particularly difficult to establish. In the first place, the so-called latent variety may be quite asymptomatic and unsuspected, and be revealed only in a survey of presumably normal, young people. Secondly, it may give rise to vague constitutional disturbances suggestive of almost any infection; thirdly, it may cause symptoms of mediastinal pressure; or it may be productive of manifestations of acute or of chronic pulmonary disease.

The type designated "latent" has been made known by the correlation of a positive reaction to tuberculin with roentgenographic evidences of pulmonary infiltration or of enlarged glands at the hilum of the lungs. The association of such findings with the symptoms of infection in individuals without other determinable cause for them establishes a clinical syndrome that has as a rule a favorable outcome. The group provocative of signs of mediastinal pressure is encountered almost without exception in children, in negroes and in primitive races and may be difficult to distinguish from neoplasms of the mediastinum. Useful differentiating signs are the less sharp contour and greater irregularity of the roentgenographic shadows of the tuberculous process, the presence of calcification in the glands at the root zone, a positive reaction to tuberculin and collateral manifestations of tuberculosis.

Frequently, Hodgkin's disease, lymphosarcoma and leukaemia cause enlargement of the mediastinal lymph nodes and although extrathoracic evidence suffices generally to differentiate these conditions from each other and from other maladies such as tuberculosis and neoplasms, the diagnostic difficulties may be insuperable. As a rule, roentgenographs are helpful, for shadows due to Hodgkin's disease have more straight lateral margins than other glandular masses, those due to lympho-sarcoma seldom extend equidistantly from both sides of the midline, and those that are cast by tuberculous nodes are more likely to show evidences of calcium. Characteristic of all is the nodular appearance and the sharp demarcation of the borders of the shadows, but in rare instances these margins are as hazy as those seen in infectious processes.

If the tuberculous disease at the root of the lung extends to invade the pulmonary parenchyma it may give rise to the clinical picture of ordinary phthisis with the signs of infiltration of the lung detected best in the interscapular region of the thorax. The X-ray in such cases shows shadows of increased density that spread from the mediastinum into the lung. Fan-shaped with hazy margins, they are of variable size and though like the changes caused by other exudative processes, their site and contour suggest their tuberculous nature. The positive response to the intradermal tuberculin test, the exclusion of other infections by special examinations and the course of the disease may be needed finally to establish its etiology.

In the evolution of pulmonary tuberculosis almost every known variety of pathological change of the lungs, pleura and bronchi may develop. It must be apparent then that to know pulmonary tuberculosis is to know all diseases of the lower air passages, and that to discuss completely the differential diagnosis of phthisis would demand broad knowledge and much time. Let it suffice now to consider some of the more usual states that tax the physician's skill.

Chronic empyema, especially the type in which the purulent exudate is limited to a relatively small area may be mistaken for phthisis. Ordinarily, the more septic type of fever, the chills, sweats, pallor, clubbing of the fingers, anaemia, leucocytosis and local signs suffice to identify the condition and thoracentesis with or without fluoroscopic guidance proves the presence of the pleural exudate. With proper roentgenographic technic, it is possible to differentiate a loculated pleural exudate from a pulmonary lesion. In patients with symptoms of broncho-pulmonary disease, examination of the chest made by the postero-anterior projection of the rays may disclose what appear to be areas of infiltration within the substance of the lungs, whereas projection of them tangentially to the fissures in the "Kreuzhohlstellung" may demonstrate oval, circular or linear, sharply bordered shadows in the interlobar fissures, in the trough of the diaphragm or along the mediastinum indicative of encapsulated pleural fluid.

Encapsulated pleural transudates may likewise be mistaken for infiltration of the lung and require like measures to differentiate them from parenchymal disease.

Occasionally, large non-encapsulated pleural exudates or transu-

dates that develop in patients with a fixed mediastinum are responsible for physical signs typical of pulmonary consolidation. The resistance to the pleximeter finger, and the obscuration of the costophrenic angle may be the only data to suggest the presence of fluid and in every doubtful case thoracentesis is justified to establish the anatomical condition.

Ordinarily, pulmonary suppuration can be recognized readily. It is so often the sequel of a surgical operation, the aspiration of a foreign body, of pneumonia, of a neoplasm or of a thoracic trauma that the history alone is strongly suggestive. Then, too, the area involved is more often basal than it is in tuberculosis, the systemic disturbances and anaemia are more profound within a shorter time, hypertrophic changes in the bones occur more regularly and tubercle bacilli can not be demonstrated in the sputum even when the search is made properly and repeatedly. The attempt to distinguish between an abscess and saccular bronchiectasis may be futile unless contrast roentgenography is utilized.

Saccular bronchiectasis tends to be bilateral and predominantly basal but now and again it involves only the bronchi of an upper lobe. In the latter event, especially, an erroneous diagnosis of ulcerative phthisis may be made unless due regard is given the relative mildness of the constitutional reaction and the persistent absence of tubercle bacilli from the abundant purulent sputum. The roentgenographic changes, without or after the intratracheal instillation of lipiodol, are not always etiologically conclusive, inasmuch as dilatation of the bronchi develops in many cases of advanced pulmonary tuberculosis. Confusion may be avoided if it be remembered that in the early stages of primary bronchiectasis and in certain types of that disease the sputum is not abundant and that in any event the constitutional reaction is usually mild except during acute episodes of broncho-pneumonic reaction.

Chronic non-tuberculous bacterial infections of the lungs show a predilection for the inferior half of the broncho-pulmonary tract. They may be confused with tuberculosis if they involve the upper lobes as well or if the tuberculous lesion is solely or predominantly a basal one. In the former event the history, the lack of sensitiveness to small doses of tuberculin intradermally injected, the absence of

tubercle bacilli from the sputum and the constant presence of other pathogenic organisms in pure cultures of the washed respiratory secretions are the essential diagnostic criteria. In the latter case, the more severe constitutional reaction, the demonstration of Koch's bacillus in the sputum, the positive reaction to tuberculin, the leucocytic formula and the signs of tuberculosis elsewhere in the body establish the proper conclusion.

Chronic bronchitis really should cause no diagnostic dilemma. More prone to affect individuals in middle or old age, secondary often to another pathological state, the cardinal symptoms of it are a seasonal productive cough, the absence of systemic disturbances and of haemoptysis, and the presence of dyspnea. The characteristic physical finding is the presence of rhonchi and moist râles distributed bilaterally and without evidences of parenchymal change other than perhaps a prolongation of expiration due to the usually associated emphysema. If, in addition to a careful general survey, roentgenography is utilized and examinations of sputum are made, there should be no reason to confuse bronchitis with pulmonary tuberculosis.

Paroxysmal dyspnea, although not usual in pulmonary tuberculosis, may occur or true asthma and phthisis may coexist. Therefore, even if a syndrome seems to be typical of bronchial asthma, failure to examine the sputum for tubercle bacilli and to seek other evidences of tuberculosis in addition to searching for an allergic, cardiac or infectious cause of the symptom-complex is a serious omission.

Mycotic infections of the lungs, although they occur relatively infrequently in this locality, may be diagnosed erroneously as tuberculosis. As a rule, the dermal lesions of blastomycosis, sporothricosis and coccidiosis are diagnostic indications and in actinomycosis a discharging sinus about the neck and chest point the way. These conditions as well as streptothricosis may give rise to pulmonary changes identical with those caused by tuberculosis though they tend to involve chiefly the lower lobes of the lungs. Although intradermal tests with extracts of sporothrix or of streptothrix may be helpful, it is the examination of the sputum or of the pus from accessible lesions that is needed finally to prove the nature of the infection.

So-called pulmonary miliary tuberculosis was not recognized clinically before the advent of roentgenography, by means of which many

minute, rounded shadows were demonstrated scattered throughout the pulmonary fields. In the active stage of the disease these shadows have hazy, ill-defined margins and a moderate density; in the inactive or healed stage they are sharply demarcated, very dense and sometimes appear to be calcified. Although often the individual whose film presents the former appearance appears to be acutely ill with a febrile disease, he may seem to be in relatively good health. The physical signs may be few indeed and may be limited to the presence of innumerable fine moist râles throughout the lungs that persist after or are increased by coughing, to slight fever and to tachycardia. As a rule, there are no local signs to indicate the presence of the scattered healed nodules. The diagnosis in the active stage rests on the detection of tubercle bacilli in the sputum, a marked intradermal or focal reaction to tuberculin or upon the outcome of the disease. The detection of the healed, inactive stage is an accidental one.

The condition may be simulated by the mottling of the lungs that develops sometimes in tuberculosis after an haemoptysis due to an allergic exudative reaction; by miliary abscesses, by so-called miliary bronchopneumonia, by miliary carcinomatosis and by silicosis. The differentiation of these several states rests on different criteria. Miliary abscesses are as a rule a manifestation of a general sepsis; miliary bronchopneumonia is a rare condition, affects infants chiefly and can be differentiated from tuberculosis chiefly by recovery with complete resolution of the loci of infiltration. Miliary carcinomatosis occurs less frequently than does disseminated tuberculosis—the nodules are usually larger, more rounded and less uniform in size than the tuberculous infiltrations and finally, the association with neoplastic growth elsewhere in the body will be a deciding factor. Silicosis may be suspected from a knowlege of the patient's occupation, by the fact that the shadows are most numerous in the lower lobes and show a greater tendency to coalesce.

In centers where certain mining and industrial activities lead to prolonged exposure of workers to an atmosphere laden with abrasive dust, *pneumonoconiosis* develops and is mistaken often for tuberculosis of the lungs. A correct diagnosis is important first in order that if the individual has pneumonoconiosis he may give up the occupation that

has imperiled his health; secondly, because if he has pulmonary tuberculosis, his continued contact with the noxious dust will be doubly hurtful to him; and thirdly, because he will be an added hazard to his fellow-workers, since the pneumonoconiotic is particularly susceptible to tuberculosis.

Uncomplicated pneumonoconiosis differs clinically from phthisis chiefly in that constitutional manifestations are mild or lacking and dyspnea is marked. Roentgenographically it causes early widening of the mediastinum especially to the right, and later bilaterally distributed linear and nodular shadows indicative of fibrosis of the lungs. These evidences of scarring are more or less equal on the two sides, are more evident in the lower half of the lungs and are associated as a rule with evidences of thickened pleura. A valuable differential datum is the absence of any signs of destruction of tissue, but often a correct diagnosis can not be made unless there is a history of prolonged occupational hazard. Acutely developing cases of this condition have occurred among the makers of silver polishes in which silica and soap are combined, and an especial variety has been described in workers in asbestos. *Asbestosis* has a symptomatology more like tuberculosis—cough, haemoptysis, emaciation—but causes more marked dyspnea and examination with the X-ray shows a very fine fibrosis of the pulmonary fields. This condition can be identified positively by the demonstration in the sputum of the characteristic golden yellow asbestos-bodies.

Massive pulmonary fibrosis may be a sequel to almost any disease in which the lungs are involved. The tuberculous and the non-tuberculous types may differ in the early stages but ultimately present identical pictures. The tuberculous variety starts near the apex, whereas fibrosis of other genesis has its origin generally at the root or in the lower lobe. If non-tuberculous scarring of an upper lobe occurs and is associated with saccular bronchiectasis, it may be most difficult to distinguish it clinically from healed tuberculosis with the formation of a cavity. The essential features of massive unilateral fibrosis are a history of chronic cough and dyspnea associated with marked retraction of one side of the chest, displacement of the mediastinum toward that side, and evidences of increased density of the homolateral lung.

Dilatation of the bronchi develops so regularly in cases of massive fibrosis of the lungs that paroxysmal cough, abundant sputum and digital clubbing indicate that the complication is present.

Atelectasis may affect small or large areas of the lungs. Due to an interference with the aeration of the alveoli, it may result from obstruction to the air passages or to compression of the lungs from without. So-called massive atelectasis should be recognizable as a rule with little difficulty, for the retraction and immobility of one side of the chest, the elevation of the homolateral leaf of the diaphragm and the displacement of the heart and mediastinal structures toward the affected side are pathognomonic. Pneumonic processes do not dislocate the mediastinum and pneumothorax or effusion push it toward the normal side. Lobular atelectasis, however, is detected less readily and there is no general appreciation of the frequency with which it occurs. It is widespread sometimes as a temporary residue of massive collapse and may then be mistaken for bronchopneumonia, tuberculous or non-tuberculous. Areas of atelectasis may develop in pulmonary tuberculosis as a result of bronchial occlusion by necrosis of the mucosa, by blood within or tubercles without the lumen of the tube, or as a result of peribronchial fibrosis, and the resultant signs may give a false estimate of the extent of the underlying lesion or lead to an erroneous diagnosis of fibrosis. The rapid variability of the physical signs furnishes the diagnostic clue but a bronchoscopic examination may be needed to determine the exact physical state.

The recognition of small *bronchial or pulmonary tumours* may be impossible without intensive study. At times they cause only symptoms of irritation of the vagus but more characteristically they are responsible for dyspnea, polypnea, paroxysmal cough, thoracic pain and tenacious sputum that contains blood. Hoarseness, unlike that due to tuberculosis, results from paralysis of the nervus recurrens. The physical signs of a small growth may be so few and equivocal that often, were not the X-ray and the bronchoscope available, a correct clinical diagnosis would be impossible. In brief, atypical signs in an area of the chest not unusually involved, especially if they are associated with evidences of bronchial narrowing or stenosis, call for endobronchial inspection. Passage of the bronchoscope only or the removal of a bit of tissue for a biopsy may suffice to relieve obstruction to the inflow of

air and demonstrate that what had seemed to be a large mass was a small tumour masked by atelectasis. This determination may have a vital influence on prognosis and treatment at the same time that the procedure reveals the presence of the growth.

Tumours of the large bronchi may simulate consolidation near the hilum, may cause atelectasis thought wrongly to be fibrosis or may spread to form infiltrated areas with excavation mistaken for tuberculous or pyogenic abscesses. So, too, the nodular type of *primary carcinoma of the lung* may cause symptoms and signs similar to those of pulmonary suppuration or tuberculous vomicae. The presence of metastases in other parts of the body especially in the regional lymph nodes, pleura, brain, bones, etc. is helpful diagnostically. The absence of tubercle bacilli from the sputum may exclude tuberculosis but their presence need not rule out neoplasm, as the two diseases may coexist.

Metastatic tumours of the lungs develop most often in cases of hypernephroma, mixed tumours of the testes, carcinoma of the prostate, thyroid and mammary glands. They may be quite latent, or give rise to few symptoms or signs. If they involve the pleura, pain and an effusion, often haemorrhagic, develop. Roentgenography of the chest shows round or irregular nodules usually distributed bilaterally and in the lower lobes like those due to inflammatory processes but as a rule more sharply outlined. If they are limited to the mediastinum such shadows may be confused with tumours primary there. Lymphangitis carcinomatosa may resemble pneumonoconiosis and is identified chiefly by the clinical history and if Hodgkin's disease involves the lung and causes a pneumonia-like change there, the enlarged lymph nodes, remittent fever, dermal manifestations, lymphopenia, eosinophilia, negative sputum and the examination of a node establish the true nature of the condition.

A number of types of acquired *syphilis of the lung* have been described, but gummata and induration are conceded to be the more usual lesions. The former vary in size from minute nodules to large tumours that may caseate and be replaced by dense fibrous tissue. Large bands of scar tissue are formed that radiate from the hilum particularly into the lower lobes, cause marked deformity of the intrathoracic contents, traction or compression of bronchi and bronchiectasis. Vascular changes may occur likewise and give rise to circulatory disturbances.

Although there are no symptoms characteristic of late syphilitic infection of the lungs, certain manifestations are considered to be suggestive. Recurrent haemoptyses and protracted fever are usual but the general health remains good. The lesions are situated usually in the middle and lower parts of the lungs, there is dullness with suppression of the respiratory sounds and few râles are heard. Thus, although the site of the disease and the absence of tubercle bacilli from the sputum speak against ulcerative tuberculosis the symptoms may suggest it.

Tracheo-bronchial syphilis is encountered now and again but in our experience syphilis of the parenchyma of the lung is a very rare occurrence. It is not justifiable to conclude that a positive Wassermann reaction establishes the luetic etiology of an associated pulmonary focus of disease nor is a favorable response to specific therapy necessarily decisive, for diseases due to treponemata other than the treponema pallidum may be influenced similarly by antiluetic measures. On the whole, the presumption of syphilis of the lung is strong if antisyphilitic treatment is followed by the healing of a pulmonary lesion in an individual who has a history of syphilis, a positive Wassermann reaction or evidences of syphilis elsewhere in the body. The demonstration of the treponema pallidum in the sputum is positive evidence too seldom obtained to be of value.

Even though one were informed of all the manifestations of the many diseases of the lower respiratory tract and although he had skill to detect them, he would still find instances in which physical examination alone would fail to discover even extensive anatomical faults. These premises admitted frankly and without embarrassment by every experienced clinician emphasize the universal need to utilize accessory diagnostic methods before excluding a nidus of broncho-pulmonary disease, particularly if subjective disturbances have indicated the presence of one.

The best examiner is the one who is aware of the limitations of his art.

FRANKLIN PAINE MALL

HENRY E. SIGERIST

In a letter written in the '80's, Mall said, "America is far back of the world in many respects, and it will probably take her a century to find it out." It took less. A few decades later, American medicine had caught up with Europe, and was beginning to play an increasingly important part in world medicine. This tremendous development was due to a group of men who, trained abroad, transplanted research methods in American soil, developed them in their own way, and who first of all created the atmosphere and spread the enthusiasm for medical research without which no real progress is conceivable. Cushing's Osler and MacCallum's Halsted have revealed to a larger public the life and work of two of these medical leaders. Dr. Simon Flexner's biography of Dr. Welch will undoubtedly throw new light on this important phase in the development of American medicine. One of the most distinguished men in this early Hopkins group was certainly Franklin Paine Mall; and yet, to the general public, he probably is one of the least known of the group. He led a retired life, devoting his entire time to his work, and to his students. There are fewer stories told about him than there are about Osler and Welch. The more welcome, therefore, is the fascinating biography that Dr. Florence Rena Sabin has devoted to her teacher.[1]

Franklin Paine Mall was born in 1862 on a farm in Belle Plaine, Iowa. He entered the medical department of Michigan University in 1880, and went through the medical curriculum customary at the time. Medical education was in poor condition then. The curriculum consisted mostly of an annual course of lectures that was repeated every year. And yet a young man who knew what he wanted could get a lot out of these medical schools. After graduation in 1883,

[1] Florence Rena Sabin: Franklin Paine Mall, the Story of a Mind, Baltimore, The Johns Hopkins Press, 1934, 342 pp. Florence Rena Sabin: Biographical Memoir of Franklin Paine Mall, 1862–1917, National Academy of Sciences of the United States of America, Biographical Memoirs, vol. XVI, Third Memoir, Washington, 1934.

Mall went to Germany, then the leading country in medicine. He started his work in Heidelberg; but then, in the fall of 1884, he went to Leipzig, and there, like so many young American students, he came under the spell of His and Ludwig. What he found in Leipzig was entirely different from what he had experienced in Michigan. Laboratories provided splendid opportunities for research, and he found first of all men who considered it their mission to train, not only physicians, but scientists. Mall knew what he wanted. He chose the men with whom he wished to work; he went to the professor of anatomy, William His, who was the outstanding embryologist of his time. From the beginning His was interested in Mall. He started him on a scientific problem, suggesting that he work on the development of the neck region in the chick. It was a difficult problem, and His did not help his students very much in their work. His method was to let them alone, but he was always ready to discuss their results. Mall was started, and the longer he stayed in Leipzig, the more absorbed he was by the general current of scientific life.

In the spring of 1885 Mall entered Ludwig's laboratory, then the breeding-place, not only of physiology, but of experimental medicine at large. When Dr. Welch, himself a student of Ludwig, heard that Mall was given an opportunity to work in the laboratory, he wrote, congratulating Mall, "It is certainly the ideal and best thing for you. I consider it in itself an education to come under the influence of such a man as Ludwig, to learn his way of looking at and attacking problems in physiology. I think that no better training is to be had than that obtained from Ludwig." Mall started some research on the villus of the intestine, and lived for a year in very close contact with Ludwig. An enduring friendship resulted. Throughout his life, Mall felt more than a pupil of the great master. He felt like a son to him. The letters of Ludwig to Mall, reproduced by Dr. Sabin, both in the German original and in English translation, are gems. They reveal Ludwig in his warm-heartedness, and enthusiasm for science, which everybody who came in contact with him felt. Even we who have learned physiology, not from Ludwig himself but from his students, have sensed his great personality, which was still alive in his students, and has been passed on through the generations.

In 1886 Mall had to go back to America. It was time to think of

making a living; but the year spent in Germany had been decisive in Mall's career. He was determined to give up easy opportunities for making a living. In a letter to his sister he wrote, "My aim is to make scientific medicine a life-work. If opportunities present, I will. This has been my plan ever since I left America, and not until lately, since having received encouragement, have I expressed myself. I shall no doubt meet many stumbling-blocks, but they are anticipated."

When Mall returned to America, the Johns Hopkins Hospital was being built. It was to become the nucleus of a medical school that was to be a new venture in American medical education. A school devoted, not only to teaching, but primarily to research. In 1884, Dr. Welch had been appointed to the chair of pathology, and long before the medical school was open, he began giving courses, grouping able young men, and giving them instruction in pathology and experimental medicine. From 1886 to 1889, Mall was a Fellow in pathology, and then Assistant under Welch. He had the best opportunity for research that America could offer at the time, and was able to continue the work begun in Germany; but the position was not permanent. So he was glad to accept a chair at Clark University in Worcester, Massachusetts, which was organized with great expectations in 1889. It was the chair of anatomy. Mall could just as well have filled in a chair of physiology or pathology. Yet it was not by accident that he was finally settled in the anatomical field. His first inclinations and his first research had been in this line, and American medicine was to benefit by his entering anatomy definitely. Ludwig may have felt somewhat disappointed. In a perfectly delightful letter he wrote, "Mein Gott, er ist nun Anatom; von uns armen Physiologen will er nichts mehr wissen. Nun Gott befohlen. Ihr alter und getreuer Ludwig."

The three years spent at Worcester were busy and fruitful years. Here Mall completed one of his most important contributions, the discovery of the vasomotor nerves of the portal vein. The new university, however, did not hold what it had promised, and in 1892 Mall accepted the chair of anatomy at the University of Chicago, where he thought that he would be able to realize his ambition, "to see a complete department of anatomy existing . . . that is, one in which all the special divisions of the subject are represented." However, things

were developing slowly, and in the meantime, the Johns Hopkins School of Medicine was getting ready to open its doors. And when the chair of anatomy was offered to Mall, he accepted in April, 1893. He wrote to His, "I am inclined to go to Baltimore, because I do not believe that we can equal Johns Hopkins before five years. The ideals and plans at Baltimore are very high. It is the first time that an American institution has placed a medical department on so high a scale and in addition the professors receive more freedom than in many German universities. At Chicago there is much to be done and I have always feared that in order to gain the same high place in Chicago at least five years of my time would have to be devoted to organization."

From 1893 to the year of his death in 1917, Mall headed the department of anatomy at the Johns Hopkins University. He was given the opportunity to build up what seemed to him the ideal department, and to set an example for the whole country. He trained generations of anatomists. About twenty-five chairs have been filled from his department. He was an inspiring teacher, living with his staff, guiding it. The task in the beginning was by no means easy. An anatomical laboratory had to be built first. Methods of procuring bodies had to be worked out. But after a year's time, Mall could resume his research, and the ensuing years were extremely fruitful. Dr. Sabin's bibliography includes 104 titles. Mall's work extended over many fields. He determined the origin of the thymus, discovered the vasomotor nerves of the portal vein, developed a theory of structural units of organs. He determined the nature of circulation through the spleen, directed studies on the development of the vascular and lymphatic systems. With his great collection of human embryos, he laid broad foundations for the study of normal and pathological embryology.

If Mall had achieved nothing else, it would have been the work of a full life-time. But besides his anatomical research, Mall became a pioneer in medical education. He knew the German system, and he had learned to appreciate it. But he was aware of its deficiencies. Great credit must be given to the founders of the Hopkins Medical School for having transplanted the spirit of the German university to this country, without copying the external forms of the German university curriculum. The European universities were founded in the

Middle Ages, and although they had developed a new spirit from the 18th century on, they still preserved institutions that had outlived themselves. Mall had attended the lectures of His, but he was convinced that anatomy has to be learned, not by lectures, but in the dissecting-room. He advocated what he called the inductive method of learning. He loathed the idea of lecturing, and wanted the students to find their own way, a method that quite a few of them resented in the beginning. Mall's teaching was "elbow-teaching." After a few hours spent in his laboratory in the early morning, he went to the dissecting-room, asking the students questions that need not necessarily be anatomical.

I do not quite agree with Dr. Sabin, in her statement that the German system of teaching anatomy is rigid. To be sure, endless lecture courses are offered to the students, most of them being required courses. However, the fact that a course is required means only that you have to register and pay for it. You still have the academic freedom of skipping them. I don't think that I have attended more than ten anatomy lectures in my life, although, according to the curriculum, I should have heard hundreds of them. But I spent the afternoons of three winter semesters, fifteen months all in all, in the dissecting-room. I quite agree that the German curriculum, particularly in anatomy, is old-fashioned, and that Mall's method is far superior. The German system, however, with its academic freedom, makes the student responsible for his education, even more than is the case here. While I was in Germany, I always advised my students to disregard the official curriculum, and to skip all the classes that did not have much to offer. Another factor that has to be taken into consideration in comparing the conditions in this country with those in Europe, is the size of the classes. In this country, we have the great advantage of having small classes, while in a large university in Europe, the anatomist may have to teach three hundred or more students at one time. Mall had his European predecessors in von Baer and Döllinger, who early recognized the nonsense of offering endless lecture courses to students. They could not realize their ideas in Europe, while Mall, who was creating in a more or less virgin soil, had the possibility of instigating a new method of anatomical teaching.

Another contribution of Mall's to medical education was the so-

called concentration system, generally adopted today at the Hopkins. Instead of rushing the students from one department to another, the system makes the students work for a definite period of time in one department, so that they can live the life of the department, in close contact with the staff. Research by students was encouraged by Mall, who wanted to make the writing of a dissertation an elective subject. Mall's leading idea in all his reform work was the self-education of the student, an idea that was revolutionary in this country. If, at Hopkins, the required hours have been cut down to a minimum, and the students are encouraged to take elective courses according to their inclinations, this is largely due to Mall's initiative. And finally, Mall was instrumental in the organization of the full-time system.

Besides his research and educational work, Mall took an active part in the development of the Association of American Anatomists, and was one of the organizers of the American Journal of Anatomy, and the Anatomical Record. In the last years of his life, he saw a long-cherished wish fulfilled—the foundation of a research institute of embryology. Mall's first paper was on an embryological subject, suggested by His. His died, but his work was carried on by Mall, and the Carnegie Institute for Embryology became a center of embryological research. Mall's activities were not spectacular, but his influence was most profound. The Johns Hopkins Medical School would not have become what it is without Mall's coöperation. He will always be remembered as an investigator, teacher, and counsellor.

We are profoundly indebted to Dr. Sabin for her splendid book, which is not only the story of a mind, but an important contribution to the history of American medicine. The book is extremely well written, the quoting of many letters brings colours into the picture. It is obvious that when you write the history of a beloved teacher, the colours may sometimes be too bright. But nobody could reproach Dr. Sabin for that. The book is undoubtedly a most valuable contribution to the history of medicine, and a splendid tribute to a great man.

THE METABOLISM OF ISOLATED SURVIVING TISSUES FROM ANIMALS RENDERED HYPERTHYROID WITH THYROXINE[1]

DONALD McEACHERN, M.D.

From the National Institute for Medical Research, Hampstead, and the Cardiographic Laboratory, Johns Hopkins Hospital and University, Baltimore

It appears to be an established fact that thyroxine exerts its final effect upon the metabolic processes of the living cell, either directly or through the agency of the intact organism. In previous communications it has been shown that the hearts of hyperthyroid animals, when isolated from the body, continue to beat at a greatly increased rate (27) and that such tissues have an enhanced oxygen consumption (29). It seemed important therefore to determine whether other representative tissues from hyperthyroid animals preserved an increased metabolism after isolation from the body, and to study the mechanism involved if such an increase were found. Since the inception of this work a number of reports have appeared which clearly show that an increased oxygen consumption is demonstrable in various tissues removed from hyperthyroid animals. These contributions will be referred to later in this report.

The present study confirms the fact that marked increases of oxygen consumption are demonstrable in tissues isolated from hyperthyroid animals and deals with a comparison of these changes to the glycolytic capacity of the tissues and to the metabolism of the intact animal. In an attempt to determine the mechanism of these changes, a study has been made of a number of substances which affect various enzyme systems in the living cell. These substances include methylene blue, cyanide, iodoacetic acid, fluoride and iodide. An effort has also been made to reproduce, in acute experiments on normal tissues, the characteristic increase of O_2 consumption by the addition of muscle enzyme

[1] Under a Fellowship of the National Research Council, Washington, D. C., and a Jacques Loeb Fellowship and a Bingham Fellowship of the Johns Hopkins Hospital and University, Baltimore.

extracts from hyperthyroid animals. Finally, a study has been made of the ability of tissues from normal and from hyperthyroid animals to oxidize various substrates *e.g.*, sodium d-lactate, sodium pyruvate, sodium succinate and dihydroxyacetone. The significance of the results will be discussed later.

LITERATURE

The literature appearing to 1931 has been reviewed in a previous paper (29). Although at that time the bulk of evidence pointed to a direct involvement of the metabolism of tissue cells in hyperthyroidism, there were nevertheless some conflicting claims. Thus Dresel (11) using the Warburg method found large increases (*ca.* 200 per cent) in the oxygen consumption of liver and moderate increases (*ca.* 25 per cent) in kidney tissue at certain periods following a single subcutaneous injection of 1.0 mgm. thyroxine to the animals. Anselmino, Eichler and Schlossmann (4), using the same technique, were unable to demonstrate an increased respiration in hepatic, renal or splenic tissues taken from rats that had been similarly injected with thyroxine.

More recently a number of observers have demonstrated an increased tissue respiration with certainty. Hopping (22) has reported an increase of from 150 to 190 per cent in the respiration of alligator's red blood cells several days after administration of thyroxine to the animals. Gerard and McIntyre (19), working with tissues from thyroid-fed dogs, found increases of 25 per cent in the respiration of liver, auricle and vagus nerve. The oxygen consumption of slices of thyroid gland was decreased 30 per cent as the result of feeding thyroid. These experiments were carried out in serum with NaOH in the vessel insets. Robles (43), studying the tissues of white mice, found an increased oxygen consumption in kidney, liver, lung and ear after prolonged feeding with a substance containing dried thyroid gland and an addition of anterior pituitary substance.

Hicks (21) found an increase of 20 per cent in the respiration of rat muscle tissue following administration of thyroxine and a decrease of 33 per cent following thyroidectomy. The estimations, however, were made on finely minced tissue and the thyroxine was administered only weekly in doses of 7 mgs. over a period of 6 weeks. Ebina (14) in a careful and complete study found a considerable increase in the respiration of hepatic and renal tissues of rats following multiple injections of thyroxine.

Dye (12), using muscle strips from young dogs, found a decrease of 25 per cent in the O_2 consumption of these tissues following thyroidectomy, and an increase of 50 per cent following the daily administration of thyroxine to the animals over long periods. The muscle strips were prepared according to the method of Richardson, Shorr and Loebel (40). Meyer, McTiernan and Aub (31) noted increases of 20 per cent in the respiration of slices of liver from hyperthyroid mice. These changes paralleled the increases of the total metabolism of the

animals. It is interesting that the metabolism of transplanted mouse tumors removed from the same animals showed, if anything, a decreased respiration. Reiss, Hochwald and Druckrey (38) found large increases in the oxygen consumption of hepatic and renal tissue from rats following the repeated administration of thyroxine. Similar increases of tissue respiration were obtained following injection of an anterior pituitary extract containing the thyreotropic hormone. Under similar circumstances, sarcoma tissue removed from rats showed no alteration in its metabolism. The authors noted that, following the administration of thyreotropic hormone, increases of tissue metabolism were obtained which equaled the maximal increases demonstrable after prolonged administration of large amounts of thyroxine. They observed that theoretically the amount of thyroxine that could be mobilized from the thyroid gland under such circumstances (*ca.* 0.1 mgm. at most) would be totally ineffective in producing an increase of tissue respiration if administered parenterally in the form of crystalline thyroxine. It is interesting that maximal increases of tissue respiration were obtained within 2 hours following a single injection of anterior pituitary extract.

From the above mentioned work and the experiments reported below, there seems to be little doubt that the metabolic changes in hyperthyroidism occur directly in the tissues without the necessity of assuming that the central nervous system acts as an intermediary. The negative results obtained by Anselmino, Eichler and Schlossmann (4) were doubtless due to the fact that the animals had not attained a sufficient degree of hyperthyroidism. In 4 animals on which total metabolism determinations were made, the increases were only from 11 to 20 per cent.

Since the publication of Haffner's paper (20) increasing emphasis has been laid (16, 1, 25 and 14) upon an increased anaërobic breakdown of carbohydrate as the fundamental mechanism in the metabolic changes of hyperthyroidism. According to Haffner's hypothesis, the increase of oxygen consumption is a secondary phenomenon due to the necessity of the organism to oxidize or resynthesize the increased metabolites. The evidence for this belief has been reviewed by Ebina (14) and is discussed in full in another place (3). It is important, therefore, to know whether an increase of anaërobic glycolysis accompanies and parallels the increases of oxygen consumption observed in isolated tissues from hyperthyroid animals.

Anselmino, Eichler and Schlossmann (4) and Dresel (11) found an increased anaërobic glycolysis in renal tissue from hyperthyroid rats but no increase of

glycolysis in hepatic tissue. Ebina's (14) protocols appear to show an increased glycolysis in renal but no constant change in liver tissue. No changes in aërobic glycolysis were observed by Ebina in either tissue. Reiss, Hochwald and Druckrey (38) found no constant increase in the anaërobic glycolysis of hepatic and renal tissue from hyperthyroid rats. Meyer, McTiernan and Aub (31) have reported an increase in the anaërobic glycolysis of hepatic tissue from hyperthyroid mice. Buell, Strauss and Andrus (7) could demonstrate no increase in the rate of formation or the amount of lactic acid formed by autolyzing skeletal and cardiac muscle pulp from thyrotoxic rabbits and cats. It is of interest that artificially implanted tumor tissues which have such a high glycolytic capacity show no increase of respiration or of aërobic or anaërobic glycolysis following their removal from host-animals that have been given active thyroid products over long periods (31, 38).

EXPERIMENTS WITH THYROXINE IN VITRO

The evidence that thyroxine can exert its effect upon tissues *in vitro* is much less conclusive, and most workers have obtained completely negative results in "acute" experiments not exceeding several hours of observation. The literature on this subject has been fully discussed by Trendelenburg (48) and by Kendall (24). Better evidence of positive effects from thyroxine on tissues *in vitro* has been obtained by the prolonged exposure of tissue cultures to the hormone. Thus Markowitz and Yater (28) found that explanted, heart-cultures from chicken embryos, when suspended in a medium containing thyroxine, exhibited a great increase in the rate of pulsation after a latent period of from 6 to 12 hours. Paal (36) found increases of over 200 per cent in the respiration of slices of thyroid gland that were suspended in serum and thyroxine for a period of 24 hours. The changes began to make their appearance after several hours of exposure to the hormone.

In the work reported below, no experiments have been performed with thyroxine *in vitro*. A large series of such experiments performed previously on isolated hearts and auricles (27) failed completely to show that thyroxine can produce its characteristic effects during the course of "acute" experiments.

GENERAL METHODS

Healthy, male, white rats of from 160 to 240 grams were used in all experiments. In some comparative experiments litter mates were used. The animals were fed daily on a synthetic diet of the following composition:

Rice starch	60 grams
Casein	30 grams
"Bemax"	5 grams
Cod liver oil	3 cc.
Salt mixture	7 grams
Water	105 cc.

The salt mixture was composed as follows: NaCl, 21 grams; Mg_2SO_4, 66 grams; $NaH_2PO_4 \cdot H_2O$, 42 grams; KH_2PO_4, 114 grams; calcium acid phosphate, 65 grams; ferric citrate, 24 grams; calcium lactate, 73 grams. Water was given ad lib.

Hyperthyroidism was induced in a few instances by mixing B.D.H. Thyroideum Siccum (of known thyroxine content) with the diet and weighing the food uneaten. In most instances thyroxine (LaRoche) was injected subcutaneously, the crystalline substance being dissolved (2 mgm. per cubic centimeter) before use, in N/100 KOH.

Save in the experiments detailed in table I, hyperthyroidism was induced by a series of daily injections of thyroxine. Experience has shown us (see table III) that considerable increases of metabolism (30 to 50 per cent) are present after 3 or 4 days of such treatment and large increases (60 to 110 per cent) at the end of a week. The doses varied from 2.5 to 30.0 mgm. per kilogram of body weight. There is little advantage, however, in giving very large doses, since the metabolism does not rise appreciably higher or faster than it does with the smaller doses. The important factor seems to be the fairly frequent (*e.g.*, daily) injection of the substance. A standard daily dose of 5.0 mgm. per kilogram was therefore given to most of the animals. Under this régime the animals lose weight rapidly; tachycardia, tachypnoea and asthenia set in, and finally death occurs if the injections are continued over a long enough period.

The basal metabolism of the animals[2] (fasted 16 to 18 hours) was measured by the method of Richards and Collison (39) and carried out in detail as described by Gaddum (18). Determinations were made daily until a level was obtained and the animal was then sacrificed for study of the metabolism of its isolated tissues, or in other instances administration of thyroxine was commenced at this point and the animal sacrificed some days later when its metabolism was greatly increased. When a comparison was made between the total metabolism of an animal and the metabolism of its isolated tissues, the determinations of the latter were made immediately after removal of the animal from the metabolism chamber.

The metabolism of isolated tissues was determined by the Warburg technique (9, 49). The animals were killed by a blow on the head and were bled from the neck. The required tissues were removed and placed in appropriate phosphate or bicarbonate Ringer's solution. Thin sections (within the limiting thickness of the tissue, Warburg (49)) were prepared from liver and kidney (cortex) by slicing

[2] I am indebted to Mr. F. F. McDonald for making these determinations.

with a razor blade. Areas of muscle tissue of the required thinness were obtained from the diaphragm near its dorsal attachments. Slicing or mincing, which results in rapidly falling curves of oxygen consumption due to injury, is not feasible in dealing with muscle tissue.

Measurements of tissue respiration were carried out in phosphate Ringer solution of the following composition:

100 parts 0.89 per cent NaCl
2 parts 1.13 per cent KCl
2 parts 1.12 per cent $CaCl_2$
10 parts of an isotonic phosphate solution made up of 2.43 grams NaH_2PO_4 and 12.68 grams Na_2HPO_4 (dry weights) to 1 liter of water

This gives a final solution isotonic with rat's serum and of pH 7.4. Glucose solution (5.6 per cent) was added to make the final concentration 0.2 per cent. Measurements were made in 3.0 cc. of the above Phosphate-Ringer, under 100 per cent O_2 and with 0.3 cc. 6 per cent KOH in the insets.

The anaërobic glycolysis was measured in a solution of the following compositions:

100 parts 0.9 per cent NaCl
2 parts 1.15 per cent KCl
2 parts 1.22 per cent $CaCl_2$
20 parts 1.30 per cent $NaHCO_3$

Glucose was added to make the final concentration 0.2 per cent. Measurements were made in 3.3 cc. of the above solution. The gas mixture of 5 per cent CO_2 in N_2 was passed over 30 inches of glowing copper before use. Analysis in the Haldane apparatus following this step showed: CO_2, 5.15 per cent; N, 94.85 per cent; O_2, 0 per cent; CO, 0 per cent. The thermostat was kept at 37.5°C. ±0.005 and readings were made every 20 minutes for 2 hours. On completion of the experiment the tissues were washed in distilled water and dried to constant weight at 110°C. All figures are for dry weights of tissues and are expressed according to Dixon's modification (9) of the Warburg conventions: Q_{O_2} = mm³. of O_2 consumed per milligram tissue per hour. Q N_2/G = mm³. of extra CO_2 produced per milligram tissue per hour.

The development of 1 cu. mm. CO_2 represents the production of 0.004 mgm. of lactic acid by glycolysis.

Following each experiment the reaction of the suspension fluid in each vessel was tested with an indicator.

Experiments (*e.g.*, with methylene blue, cyanide, etc.) in which the Q_{O_2} or Q N_2/G was computed for the second hour instead of the total two-hour period, are so noted in the tables.

RESULTS

Tissue respiration following a single injection of thyroxine

Table I summarizes the changes in tissue respiration at various time intervals following administration of a single large dose of thyroxine to the animal. It will be noted that, although considerable increases (up to 60 per cent) in the respiration of kidney and liver were observed

TABLE I

Q_{O_2} of tissues from normal animals and from hyperthyroid animals after a single injection of thyroxine

Determinations based on 2-hour period. Oxygen use in mm³. per milligram (dry weight) per hour.

DATE	KIDNEY			LIVER			DIAPHRAGM			THYROXINE	
	N	H	Per cent increase*	N	H	Per cent increase*	N	H	Per cent increase*	Milligrams per kilogram	Time after injection
1932											
11/8	21.40			8.05			4.31				
11/9	21.14			8.14			4.13				
11/11	18.10			10.09			5.10				
11/11	20.40						5.24				
11/18		23.65	10		9.91	—				10	22 hours
11/16	19.68	32.24	50	8.67	16.60	60		5.35	8.5	10	44 hours
11/14	22.60	24.30	13	9.12	9.89	—	5.03	4.85	—	10	48 hours
11/17	21.80	25.35	18	10.28	11.75	13	5.58	5.39	9.3	10	68 hours
11/15	23.08	28.64	33	13.45	12.50	20	4.84	5.16	4.7	10	72 hours
11/18		30.95	44		12.90	24		4.36	—	10	90 hours
11/22	25.35	28.20	31	15.30	16.60	60	5.22	5.61	13.8	10	8 days
Mean..	21.50	27.62	28.5	10.39	12.88	24	4.93	5.12	3.9		

* Per cent increase of respiration in hyperthyroid tissue as compared with mean respiration of normal series.

in isolated instances, the mean increases were only 28.5 and 24 per cent, respectively. Muscle tissue showed no significant alteration. The increases of respiration in renal and hepatic tissue were variable and not always parallel in the two tissues; nor could they be correlated with the time of injection of thyroxine. In no instance was there an increase of the degree reported by Dresel (11) in liver tissue following a single injection of thyroxine.

Tissue respiration following multiple injections of thyroxine (see table II)

Following a series of injections of thyroxine considerable and constant increases were found in the tissue respiration. As compared with the mean of the figures obtained for the normal series, these increases averaged 46 per cent for kidney, 73 per cent for liver and 75

TABLE II

Qo_2 of tissues from normal animals and from hyperthyroid animals following multiple injections of thyroxine

Determinations based on 2-hour period. Oxygen use in mm³. per milligram (dry weight) per hour.

DATE	KIDNEY			LIVER			DIAPHRAGM			THYROXINE		
	N	H	Per cent increase*	N	H	Per cent increase*	N	H	Per cent increase*	Milligrams per kilogram	Doses	Days
1932												
12/1	23.20	33.30	40.4	9.98	16.34	70.5	4.40	6.98	48.3	10	2	5
11/29	27.30	34.00	43.3	10.32	17.05	77.9	5.36	10.20	116.3	10	3	13
11/30		29.00	22.3	11.01	18.25	90.3	6.01	8.98	90.5	10	4	14
12/10	21.40	35.80	51.0	8.36	19.68	113.7	4.42	8.97	90.5	10	5	14
12/14				10.10	15.38	60.4				10	7	17
12/16				7.25	14.70	53.4				10	8	18
12/6	23.05	33.75	42.4	9.07	14.45	50.6	4.84	9.07	92.5	15	2	5
11/24	21.65	24.10	1.6	8.85	15.25	59.0	4.10	5.05	7.0	15	2	7
11/25	21.00			8.65	14.45	50.6	3.68	5.37	14.0	15	2	9
11/26	27.70	42.55	79.5	11.55	20.15	110.0	5.30	9.85	109.0	15	2	9
12/7	24.45	33.85	42.7	10.25	15.00	56.5	4.30	9.80	108.0	15	3	6
12/8		45.55	92.2		19.04	98.5		8.93	89.5	15	3	7
Mean..	23.72	34.65	46.1	9.59	16.62	73.3	4.71	8.32	75.5			

* Per cent increase of respiration in hyperthyroid tissues as compared with mean respiration of normal series.

per cent for diaphragm. The maximum increase in kidney tissue was 92 per cent, in liver 114 per cent and in diaphragm 116 per cent. The increase of respiration varied widely in the different tissues from a given animal but in each experiment there was definite evidence of a persisting high metabolism in the tissues. Figure 1 indicates the curves of oxygen consumption obtained in a typical experiment. It will be noted that the curves are well sustained over the two hours of

observation, and that within that period there is no greater diminution of respiration in the hyperthyroid tissues than in the normal tissues.

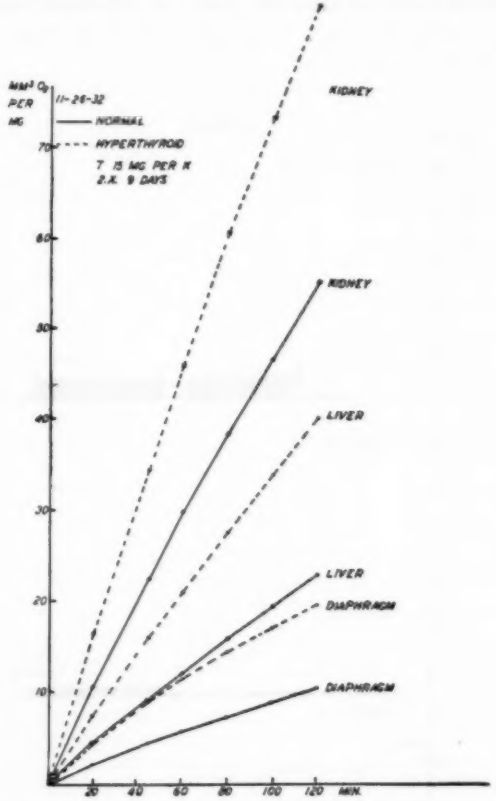

FIG. 1. METHOD OF STUDYING TOTAL METABOLISM OF ANIMALS

Measurements of tissue metabolism were made immediately following the last determination of total metabolism.

Comparison of total metabolism of animals with respiration and glycolysis of their isolated tissues

Figure 2 illustrates the method of following the total metabolism of the animals. The measurements of tissue metabolism were made im-

mediately following a series of daily determinations of total oxygen consumption.

Table III summarizes the changes in the respiration and anaërobic glycolysis of isolated tissues as compared with the total oxygen consumption of the animals.

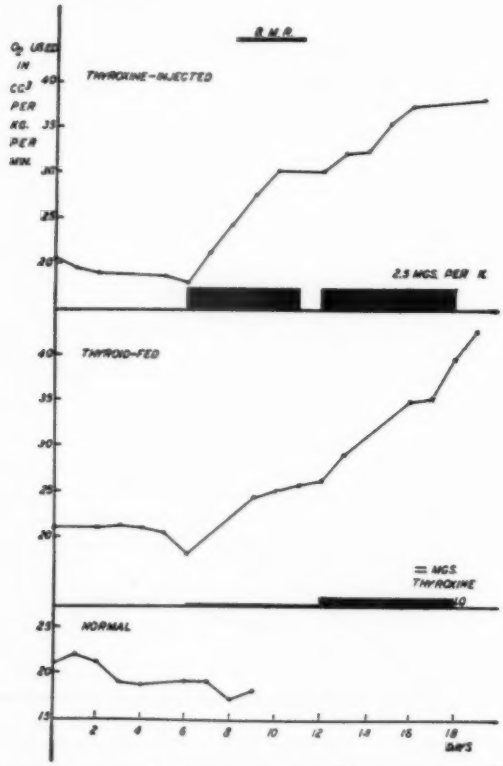

FIG. 2. TYPICAL CURVE OF OXYGEN UPTAKE BY TISSUES FROM A NORMAL AND FROM AN HYPERTHYROID ANIMAL

The mean increase of total oxygen consumption of 7 hyperthyroid animals was 89 per cent, whereas the individual tissues showed a mean increase of 57 per cent for kidney, 45 per cent for liver and 53 per cent for diaphragm. Some of the discrepancy between the figures for

total metabolism and tissue metabolism might be accounted for by the fact that there was dehydration in the hyperthyroid animals. Skeletal muscle from hyperthyroid animals contains 6.3 per cent less

TABLE III

Comparison of total metabolism of normal and hyperthyroid animals to Q_{O_2} and Q_{N_2}/G of their isolated tissues

Determination of tissue metabolism based on 1-hour period

DATE	THYROXINE			ANIMAL			KIDNEY			LIVER			DIAPHRAGM		
	Milligrams per kilogram	Number of doses	Number of days	B. M. R., cm³. per gram per hour			Q_{O_2}			Q_{O_2}			Q_{O_2}		
				Normal	After thyroxine	Per cent increase†		Per cent increase†	Q_{N_2}/G		Per cent increase†	Q_{N_2}/G		Per cent increase†	Q_{N_2}/G
Normal															
1933															
1/20				1.28			29.10			10.63			4.69		
1/26				1.11			24.62		4.72	8.54		1.16	4.42		4.36
2/15				1.31			21.40		3.41	9.87		2.32			4.21
3/13				1.13			21.00		2.68	7.26		1.04	6.13		4.18
3/8				1.02			27.10		2.82	7.70		1.16	5.37		5.43
2/22				1.26			20.92		1.35	10.53			5.59		4.42
Mean..				1.18			24.02		2.99	9.09		1.42	5.24		4.52
Hyperthyroid															
3/23	1.0*	6	6	1.17	1.84	57.2	44.90	84.0	3.92	13.80	58.6	1.63	7.13	40.3	
2/3	10.0	3	7	1.16	1.93	66.4	40.50	67.0	7.48	15.60	79.0	1.80	6.23	22.6	4.12
1/20	10.0	3	4	0.97	1.98	104.0	36.00	47.6		15.55	79.0		6.12	20.4	
3/7	2.5	12	14	1.14	2.09	83.5	37.24	53.6	4.92	12.78	47.0		8.50	67.3	3.55
1/25	20.0	4	8	1.18	2.26	91.6	34.50	42.2	7.35	11.56	33.0	4.72	8.55	68.0	3.38
3/6	2.5	11	12	1.08	2.30	113.0	35.50	45.6	4.66	10.90	25.3	1.25	10.33	103.5	4.10
2/9	0.66*	9	9	1.23	2.57	109.0	42.90	75.8	5.17	12.70	46.0	1.26	9.24	82.0	5.63
Mean..				1.13	2.14	89.3	37.77	57.2	5.70	13.18	45.0	2.13	8.01	52.7	4.16

* Grams B.D.H. thyroideum siccum by mouth per day.

† Increase above average of normal series.

water than does normal muscle (3). When the loss of weight of the hyperthyroid animals is disregarded and the total metabolism calculated on the original body weight, the mean increase of oxygen con-

sumption amounts to 73 per cent instead of 89.3 per cent. There were wide variations in the degree of increase of respiration in the various tissues in each experiment and of the respiration of the same type of tissue in different experiments.

The anaërobic glycolysis of renal tissue from hyperthyroid animals was increased 91 per cent. This increase in glycolysis bore no constant relationship to the increased oxygen consumption of the tissue or of the intact animal. There was no significant change in the glycolysis of liver or diaphragm. In view of the importance of establishing this point, further experiments were carried out in neutralized sodium cyanide (M/700) in order to inhibit any possible respiration due to oxygen present in the tissues. These experiments are summarized in table IV. Further glycolysis experiments appear in table V. In all, measurements of anaërobic glycolysis were made on the tissues of 20 normal animals and 18 hyperthyroid animals. An increase of glycolysis was found only in the case of renal tissue. The presence of an increased glycolysis in renal tissue from hyperthyroid animals has been noted by a number of workers (11, 4, 14).

Experiments with methylene blue

In view of the striking effects of methylene blue on the respiration and glycolysis of some cells it was thought important to test the response of tissues from hyperthyroid animals. It seemed possible, if the fundamental metabolic change in hyperthyroidism were due to some process of dehydrogenation leading to the production of fixed acid, that the change might be recognized by supplying a preformed hydrogen acceptor such as methylene blue. For this purpose a purified medicinal methylene blue was used in a final concentration of 0.005 per cent, 0.33 cc. of a freshly made 0.05 per cent solution being added from the side arms of the manometer vessels after readings had been taken for one hour. The readings were then continued for another hour. (It is important that pure samples of the dye be used since some products give rise to positive pressures when added to bicarbonate buffer.) Control experiments showed no pressure changes with the sample used.

The results of experiments with methylene blue are assembled in table V. It will be noted that the respiration of normal and of hyper-

TABLE IV

Comparison of glycolysis in normal and hyperthyroid tissues with and without sodium cyanide

Determinations based on 1-hour period

DATE	THYROXINE				KIDNEY			LIVER			DIAPHRAGM		
	Milligrams per Kilogram	Number of doses	Number of days	conc. NaCN	Q_{O_2}	Per cent increase*	Q_{N_2}/G	Q_{O_2}	Per cent increase*	Q_{N_2}/G	Q_{O_2}	Per cent increase*	Q_{N_2}/G
Normal													
2/17/33					27.10		3.82	9.80			4.17		4.36
2/27/33					22.80		3.52	7.09		6.80	5.25		5.16
3/10/33					24.50		3.21	8.61		1.18			3.48
12/ 6/32								7.00		1.47			
Mean.....					24.80		3.52	8.12			4.71		4.33
12/11/33				M/700			3.28			1.84			5.20
12/12/33				M/700			4.74			1.34			2.92
12/13/33				M/700			3.96			1.93			6.28
Mean.....							3.99			1.37			4.80
Hyperthyroid													
12/ 6/32	10.0	8	20					15.00	84.7	1.92			
1/27/33	10.0	4	7		34.70	40.0	10.25	9.20	13.3	1.12	6.62	40.5	3.88
2/ 2/33	10.0	7	14		45.90	85.0	2.72	17.20	112.0		6.70	42.6	5.26
3/15/33	2.5	15	22		34.10	37.5	6.58	15.15	85.6	1.52	8.20	74.2	7.33
Mean....					38.23	54.2	6.52	14.14	73.9	1.52		52.4	
12/11/33	10.0	4	6	M/700			6.35			1.82			4.31
12/12/33	10.0	6	7	M/700			5.47			0.77			5.45
12/13/33	10.0	4	5	M/700			5.70			1.27			5.17
Mean....							5.84			0.95			4.98

* Increase above average of normal series.

thyroid tissues was affected in the same way by the dye. Thus the mean Q_{O_2} of kidney tissue decreased 57 per cent in the normal series and 62 per cent in the hyperthyroid series, the mean Q_{O_2} of liver tissue

TABLE V

Effect of methylene blue on respiration and glycolysis

Methylene blue added at end of first hour and readings continued to end of second hour

DATE	KIDNEY						LIVER						DIAPHRAGM					
	Q_{O_2}			Q_{N_2}/G			Q_{O_2}			Q_{N_2}/G			Q_{O_2}			Q_{N_2}/G		
	Before	After	Per cent change	B	A	Per cent change	B	A	Per cent change	B	A	Per cent change	B	A	Per cent change	B	A	Per cent change
Normal																		
2/ 7/33	26.00	9.50	−63	4.75	10.70	+125	11.40	10.27	−10	3.62	3.62	0	3.45	4.85	+46	3.95	3.78	−4
1/26/33	24.62	11.18	−55	4.74	19.00	+301	8.54	6.08	−29	1.16	6.50	+464	4.42	5.43	+23	4.36	4.48	+3
12/ 1/32	24.20	10.07	−71	3.05	6.44	+111	10.38	8.22	−21	2.11	2.46	+19	4.90	5.51	+12	4.02	3.87	−4
12/ 6/32	24.15	9.05	−63	3.16	7.61	+141	9.84	7.30	−26	1.33	1.58	+15	5.10	5.90	+16	4.71	4.28	−9
9/27/33	19.83			2.70	10.83	+301	9.65	10.00	+4	2.88	3.68	+28	5.43	8.32	+53	3.89	3.07	−21
9/30/33	25.60	7.23	−72				10.65	10.95	+3	1.84	5.52	+200	6.75	10.85	+61	3.96	3.65	−8
9/31/33	21.30	7.23	−66	4.42	7.55	+74	11.22	10.38	−7	1.41	5.89	+318	6.14	9.36	+49	5.22	4.07	−22
12/ 7/33	29.23	18.78*	−36	1.25	3.03	+142	13.70	7.31	−5	0.49	1.25	+16	6.52	8.66	+33	3.53	2.37	−33
	24.36	10.43	−57.2%	3.44	9.31	+172%	10.67	8.84	−17%	1.85	3.81	+106%	5.34	7.37	+38%	4.20	3.70	−12%
Hyperthyroid																		
2/ 9/33	42.90	14.00	−66	5.17	6.55	+27	12.70	7.53	−40	1.26	1.60	+27	9.24	10.38	+12	5.63	4.68	−17
2/ 3/33	36.75	11.30	−69	6.60	11.12	+68	12.84	9.07	−29	1.44	1.82	+26	4.35	7.50	+70	3.51	3.41	−3
2/ 2/33	45.90	16.60	−64	2.72	5.43	+100	17.20	13.60	−21				6.70	7.90	+18	5.26	3.74	−29
12/ 8/32	44.80	17.00	−62				17.70	12.90	−27				8.16	8.88	+9			
12/ 1/33	34.40	11.79	−66				16.96	11.77	−31				6.70	7.85	+17			
12/ 6/33	34.02	13.50	−60				15.10	10.06	−33				9.61	12.20	+27			
11/25/33	38.18	20.03	−48	5.64	7.24	+28	15.37	14.93	−3	1.59	2.42	+52	9.47	15.53	+64	7.29	5.20	−29
	39.56	14.89	−62.4%	5.03	7.58	+50%	15.41	11.42	−26%	1.43	1.95	+36%	7.75	10.03	+29%	5.42	4.25	−22%

* Methylene blue final concentration 0.005 per cent.

decreased 17 and 26 per cent respectively in the two series and the mean Q_{O_2} of muscle increased 38 and 29 per cent respectively in the two series. It is to be remembered of course that in control experiments without methylene blue some diminution of oxygen consumption occurs over the two-hour period. This amounts to about 8 to 12 per cent for hepatic and renal tissue and to about 10 to 15 per cent for diaphragm, when the Q_{O_2} of the second hour period is compared with that of the first hour. The per cent decrease is no greater in hyperthyroid than in normal tissues. The decrease of respiration in kidney and liver tissue in the presence of methylene blue is probably only an apparent decrease, since there is considerable reduction of the dye. This would indicate that these tissues use some oxygen from the methylene blue in preference to molecular oxygen.

Striking increases in the anaërobic glycolysis of normal renal tissue occurred in the presence of methylene blue. These increases occurred consistently, the maximum amounting to 300 per cent (2 experiments). The mean increase for the normal series was 172 per cent. Such large increases of glycolysis were not noted in the hyperthyroid series in which the mean $Q_{N_2/G}$ in the presence of methylene blue was but 50 per cent greater than in the control period. The mean increase of glycolysis in liver tissue as a result of methylene blue was 106 per cent for the normal series and 36 per cent for the hyperthyroid series. There were, however, wide variations in the normal series, the maximum increase being 464 per cent and the minimum 15 per cent. There was no apparent difference in the effect of methylene blue on the glycolysis of skeletal muscle from normal and hyperthyroid animals, mean decreases of 12 and 22 per cent occurring in the two series, respectively. The reason for the difference between the effect of methylene blue on the glycolysis of normal and hyperthyroid renal and hepatic tissue is not apparent. It is possible that it might be associated with a depletion of tissue substrate in the hyperthyroid animals. Certainly a great diminution of liver glycogen occurs in hyperthyroid animals. Although glucose was used as a substrate in all experiments, the amount of lactic acid formed in normal tissues by the breakdown of glycogen might account for the difference.

* Methylene blue final concentration 0.005 per cent.

Experiments with sodium cyanide

In view of the fact that the normal respiration of animal tissues is completely inhibited by cyanides (2, 50) it seemed of importance to determine whether the extra increment of respiration due to hyperthyroidism was associated with some mechanism other than the iron-containing *Atmungsferment* of Warburg. As shown in table VI the inhibition of respiration in renal, hepatic and muscle tissue from normal and hyperthyroid animals was tested in M/70 sodium cyanide using phosphate buffer. Further experiments with renal tissue compare the degree of inhibition with various, lower concentrations of sodium cyanide. The cyanide solution was made up freshly before each experiment and neutralized with HCl. Alt (2) and Warburg (50) have shown, and it has later been confirmed by Dixon (10), that complete inhibition of respiration by cyanides is obtained only when the experiments are carried out in bicarbonate buffer in the presence of an atmosphere containing 5 per cent CO_2. The present experiments are therefore merely comparative, but serve to show that the extra moiety of respiration due to hyperthyroidism is cyanide-sensitive and, in the case of renal tissue, is even more sensitive than that of normal tissue.

It will be noted that with M/70 cyanide an approximately equal degree of inhibition occurred in the respiration of all tissues—in general, about 90 per cent. This inhibition was as great in hyperthyroid as in normal tissues. Renal tissue from hyperthyroid animals was, however, much more sensitive than normal renal tissue when the respiration was measured in lower concentrations of cyanide. Thus while the respiration of normal kidney was unaffected in M/1400 NaCN that of hyperthyroid kidney was inhibited by 22 per cent in M/2800 NaCN. This increased sensitivity of hyperthyroid renal tissue to cyanide was noted throughout all the other concentrations up to M/70. The respiration of hyperthyroid renal tissue in M/1400 to M/2800 cyanide was brought down more or less to that of normal kidney without cyanide and remained on a well sustained level till the end of the experiment. The determination of Q_{O_2} shown in table VI are based upon both the first and the second hour of observation, since the effect of cyanide in high concentration is not always maximal

TABLE VI

Respiration of tissues from normal and hyperthyroid animals in presence of sodium cyanide

TABLE VI

Respiration of tissues from normal and hyperthyroid animals in presence of sodium cyanide

DATE	HOUR	Q_{O_2} KIDNEY															Q_{O_2} LIVER			Q_{O_2} DIAPHRAGM		
		Conc. NaCN																				
		None	M/70	Per cent change*	M/350	Per cent change*	M/700	Per cent change*	M/1400	Per cent change*	M/2100	Per cent change*	M/2800	Per cent change*	M/3500	Per cent change*	None	M/70	Per cent change*	None	M/70	Per cent change*
Normal																						
1933																						
11/ 2	1st	21.57	5.76	−73													8.45	2.57	−70	5.84	1.86	−68
	2nd	16.43	3.08	−81													7.38	0.15	−98	5.21	0.73	−86
11/ 3	1st	21.92	4.70	−79													8.74	2.98	−66	5.81	2.38	−59
	2nd	16.00	1.75	−89													7.25	0.65	−91	4.75	0.80	−82
11/14	1st	23.83	4.52	−81	7.14	−70	9.35	−61	18.52	−22			22.60	−5								
	2nd	18.74	1.83	−90	5.93	−63	10.90	−42	20.09	+7			19.21	+3								
11/21	1st	25.80									23.39	−9	23.05	−11	22.93	−11						
	2nd	20.20									20.33	0	19.60	−4	21.02	+4						
Hyperthyroid																						
11/ 7	1st	34.40	4.79	−86													14.03	1.61	−88	9.59	1.82	−81
	2nd	23.40	2.01	−91													12.07	0.80	−93	6.48	0.71	−89
11/ 9	1st	36.95	6.28	−83													18.13	2.56	−86	11.21	2.73	−76
	2nd	30.25	1.93	−94													15.32	0.64	−96	10.98	0.75	−93
11/10	1st	34.38	3.89	−89	4.81	−86	6.05	−82	10.33	−70			20.73	−40								
	2nd	27.97	1.41	−95	2.87	−90	6.64	−75	12.46	−55			21.67	−23								
11/24	1st																					
	2nd	40.02									28.00	−30	31.32	−22	36.34	−9						

* Per cent change of Q_{O_2} as compared to Q_{O_2} of control without cyanide.

during the first hour. On the other hand, tissues suspended in low concentrations of cyanide tend to "escape" from its effect during the course of a two-hour experiment.

Experiments with fluoride and iodoacetate

If an increased glycolysis were the fundamental cause of the increased oxygen consumption in hyperthyroidism, it might be expected that the extra moiety of respiration would be inhibited by certain sub-

TABLE VII

Effect of iodoacetate and sodium fluoride on respiration of tissues from normal and hyperthyroid animals

DATE	TISSUE	Q_{O_2} SECOND HOUR													
			Iodoacetate						Sodium fluoride						
		Control	1.10^{-4}	Per cent change	1.10^{-3}	Per cent change	1.10^{-4}	Per cent change	M 0.02	Per cent change	M 0.002	Per cent change	M 0.0002	Per cent change
						Normal									
1934															
2/6	Kidney	19.94			5.26	−74	10.56	−47			15.28	−23	20.36	+2
2/7	Kidney	17.73			2.77	−84	9.50	−46			8.43	−52	14.05	−21
2/7	Liver	5.45									3.75	−31		
						Hyperthyroid									
1/17	Kidney	37.86	1.47	−96					6.12	−84				
1/18	Kidney	26.07			4.82	−82	17.97	−31			19.53	−25	28.08	+8
1/31	Kidney	23.75			0.65	−97	24.50	+3			9.25	−61	25.50	+7
1/23	Liver	8.10			6.21	−23	11.20	+26			8.61	+6		

stances that break the chain of lactic acid production. Furthermore, hyperthyroid tissues might be more sensitive to these inhibitors than would normal tissues. In table VII are assembled the results of some experiments with various concentrations of sodium fluoride and sodium iodoacetate. The solutions were freshly prepared before use and the iodoacetic acid (Eastman Kodak Company No. 1371) neutralized with NaOH. It will be noted that the respiration of renal tissue from hyperthyroid animals was inhibited by these substances no more than

that of normal tissue. In another place (30) it is noted that the administration of fluoride, of iodoacetate and of pancreatic amylase to hyperthyroid animals failed to prevent their decline and death from hyperthyroidism.

Effect of potassium iodide

There is every reason to believe that the beneficial effect of iodine therapy in human exophthalmic goiter is brought about through the medium of the thyroid gland. Some workers have claimed however that the effect takes place in the tissues of the body. It seemed of interest therefore to test the response of isolated hyperthyroid tissues to iodine. The results of a few experiments with potassium iodide

TABLE VIII

Effect of potassium iodide on tissue respiration

DATE	ANIMAL	TISSUE	Q_{O_2}				
			Control	KI 1.10^{-5}	Per cent change	KI 1.5^{-4}	Per cent change
1933							
11/21	Normal	Kidney	23.00	19.50	−15		
11/24	Hyperthyroid	Kidney	40.00	35.75	−11		
12/ 7	Normal	Liver	13.70			11.46	−16
11/25	Hyperthyroid	Liver	15.37			13.67	−11

are shown in table VIII. Concentrations of 1.10^{-5} and 1.5^{-4} were used. Such concentrations are much greater than those computed to be necessary in the human being for a maximal remission of hyperthyroidism (47). It will be noted that both normal and hyperthyroid tissues exhibited a small decrease of respiration in the presence of KI. This decrease was no greater in hyperthyroid than in normal tissues. Animals, in which hyperthyroidism has been induced by thyroid products, show no remission when iodine is administered (26, 44, 55). Similarly, there is no decrease in the tachycardia of perfused hearts from hyperthyroid animals when iodine is added to the perfusate (27).

Experiments with aqueous enzyme extracts from muscle

A curious feature in the action of thyroxine or other active thyroid products is the long latent period before evidences of their effect be-

come manifest. Thus, following thyroid feeding or the subcutaneous or intravenous injection of thyroxine, some hours elapse before any elevation of basal metabolism, of heart rate etc., occur. This has been variously attributed to the long time necessary for the absorption and incorporation of thyroxine in the tissue cells; to the slow initiation of an increased glycolysis in the tissues or to the fact that some specific organ in the body (*e.g.*, liver) may slowly break down the thyroxine

TABLE IX

Effect of muscle extract from normal and hyperthyroid rabbits on respiration of normal rat tissues

DATE	THYROXINE ADMINISTRATION			MUSCLE EXTRACT		Q_{O_2} LIVER			Q_{O_2} KIDNEY		
	Milligrams per kilogram	Doses	Days	Source of extract	Dilution of extract*	Control	With extract	Per cent change	Control	With extract	Per cent change
1934											
3/1				Normal rabbit	1:5	11.17	13.17	+18	25.40	38.80	+53
2/9				Normal rabbit	1:4	7.59	13.46	+77	20.30	31.13	+53
2/9				Normal rabbit	1:2	7.59	13.22	+74	20.30	41.20	+103
2/15	0.6	7	8	Hyperthyroid rabbit	1:4	8.11	10.73	+34	21.73	27.65	+27
2/15	0.6	7	8	Hyperthyroid rabbit	1:2	8.11	10.13	+29	21.73	32.68	+50
3/5	1.0	6	8	Hyperthyroid rabbit	1:5	12.86	13.54	+5			
3/5	1.0	6	8	Hyperthyroid rabbit	1:5 + glycogen 0.3 per cent	12.86	14.61	+14			

* Filtered extract prepared from 100 grams (wet weight) muscle pulp in 200 cc. H_2O.

molecule into a more active substance which directly affects the metabolism of other tissues.

Since it has been shown (3) that aqueous extracts from muscles of hyperthyroid rabbits decolorize methylene blue in the presence of sodium succinate more rapidly than extracts obtained from normal animals, it seemed of interest to test the effects of such extracts on the respiration of normal cells. It appeared possible that immediate increases of respiration might be obtained in normal cells by the trans-

ference of the aqueous extractives from the muscle of an hyperthyroid animal.

The aqueous extract of muscle was made according to the method of Meyerhof (32). Pairs of male white rabbits of similar weight were chosen, one of the pair being used as a control and the other injected with thyroxine 1.00 mgm. daily for 6 to 10 days until definite evidences of hyperthyroidism were apparent. The animals were killed by a blow on the head and were bled from the neck. The steps in preparing the extract are described in detail elsewhere (32). Measurements of tissue respiration were made on slices of liver and kidney from normal rats with the addition of muscle extract in varying amounts. Because of the acidity of the extract the buffer was made up with 5 times strength phosphate. Results of these experiments are tabulated in table IX. It will be noted that the increase of tissue respiration upon addition of normal enzyme extract was greater than that following addition of extract from hyperthyroid animals. In no case was there an increase of tissue respiration, due to the addition of the latter extract, of a degree that would suggest the transference of the principles which give rise to the metabolic changes in the intact muscle itself. The increases of tissue respiration which were obtained are attributed to the presence of metabolites in the extract. Such extracts contain about 1.5 mgm. lactic acid per cubic centimeter.

Experiments with sodium lactate

The experiments tabulated in table X show the relative abilities of normal and of hyperthyroid tissues to oxidize an added substrate of sodium lactate. Glucose, 0.2 per cent, was also present in the buffer solution in all experiments with lactate. For these experiments two samples of sodium lactate were used. The first was prepared by neutralization of a solution of crystalline sarco-lactic acid (British Drug Houses)[3] with sodium hydroxide. The second sample was obtained by 24-hour extraction of acidified zinc d-lactate (Eastman Kodak Company No. 2557)[4] with ether. The lactic acid obtained (94 per

[3] The British Drug Houses, Ltd., inform me that this preparation gives the following specific rotations: $[\alpha]_{D}^{20} = +1.95°$, $[\alpha]_{Hg5461}^{20} = +3.15°$.

[4] The zinc d-lactate supplied by Eastman contains 13.1 per cent or less of water. The rotation of a 4 per cent solution in a 2 dcm. tube at 25° using the green line of mercury is −0.70°.

TABLE X

Effect of sodium lactate on respiration of tissues from normal and hyperthyroid animals

DATE	HOURS ANIMAL FASTED	THYROXINE			Form and Concentration of Lactate	Q_{O_2} DIAPHRAGM				Q_{O_2} LIVER				Q_{O_2} KIDNEY			
		Milligrams per kilogram	Number of doses	Number of days		Control	Lactate	Change	Per cent change	Control	Lactate	Change	Per cent change	Control	Lactate	Change	Per cent change
					Normal												
1934																	
4/16	0				B.D.H. sodium lactate M 0.018	5.10	5.08	−0.02	0	6.13	6.06	−0.07	0	23.60	31.92	+8.32	+35
4/27	24					4.87	6.65	+1.78	+37	10.76	18.96	+8.20	+76	23.04	30.25	+7.21	+31
4/28	24					6.42	7.69	+1.27	+20	8.92	18.04	+9.12	+102	23.61	38.51	+14.90	+63
5/ 4	24				B.D.H. lactate conc. M 0.036	7.04	11.32	+4.28	+61	10.96	15.98	+5.02	+46	23.14	35.68	+12.54	+54
3/28	24				Eastman's sodium lactate M 0.018	5.28	4.46	−0.82	−15	9.30	10.43	+1.13	+12	26.22	36.32	+10.10	+38
3/17	24					5.00	5.68	+0.68	+14	6.00	7.20	+1.20	+20	21.92	23.10	+1.18	+5
					Hyperthyroid												
4/26	24	5.0	11	15	B.D.H. sodium lactate M 0.018	9.94	17.89	+7.95	+80	17.03	26.73	+9.70	+57	30.22	50.28	+20.06	+66
4/28	24	5.0	13	17		10.05	16.11	+6.06	+60	16.90	26.25	+9.35	+55	38.49	49.62	+11.13	+29
4/17	0	5.0	4	6										27.20	32.23	+5.03	+18
3/29	24	5.0	11	13	Eastman's sodium lactate M 0.018	19.30	16.70	−2.60	−13	7.30	13.90	+6.60	+90				
3/27	24	5.0	9	11		12.47	13.84	+1.37	+11	8.84	15.95	+7.11	+80	32.55	36.95	+4.40	+14

cent recovery) was boiled for 10 minutes to remove lactones and then neutralized with NaOH. Determinations of lactic acid were made by the method of Friedemann and Kendall (17) with West's (52) improved apparatus. Ninety-eight per cent of lactic acid was recovered by this method from a solution of known zinc lactate content.

Table X indicates both the percentage rise and the actual rise of Q_{O_2} in various normal and hyperthyroid tissues, due to the addition of sodium lactate. The actual rise of Q_{O_2} is probably of most significance, since it presumably indicates the actual amount of added lactate that the tissue is able to oxidize. Only experiments on fasting animals are considered in the discussion below (33).

It will be noted that muscle tissue from hyperthyroid animals oxidized about 5 times more B.D.H. sarco-lactate than did normal muscle. Hyperthyroid muscle tissues oxidized only twice as much of the Eastman salt as did normal tissues. In one experiment with normal diaphragm, B.D.H. sarco-lactate M/0.036 caused an increase of oxygen consumption $2\frac{1}{2}$ times that obtained by the same salt in M/0.018 concentration.

In the case of liver, however, the B.D.H. sarco-lactate gave rise to large increases of oxygen consumption in both normal and hyperthyroid tissues and the increases were no greater in the latter tissues than in normal tissues. Addition of Eastman salt caused small increases in the respiration of normal liver and large increases in that of hyperthyroid liver, the latter oxidizing about 6 times more of the Eastman lactate than did normal liver tissue. The increase of respiration in normal liver tissue was not greater in M 0.036 sarco-lactate than in a concentration of M 0.018.

The response of renal tissue to added lactate varied within wide limits. As will be seen, however, from table X, there is no reason to believe that hyperthyroid renal tissue oxidizes less lactate than does normal kidney tissue. The same can be said of liver tissue. Muscle tissue from hyperthyroid animals has a much greater capacity for oxidizing lactate than has normal muscle tissue. In view of the conception that an increased glycolysis might be the cause of the high oxygen consumption in hyperthyroidism, these results are of considerable interest. Their significance will be discussed later.

TABLE XI

Effect of sodium succinate, sodium pyruvate and dihydroxyacetone on respiration of tissues from normal and hyperthyroid animals

DATE	HOURS FASTED	THYROXINE			SUBSTANCE ADDED	Q_{O_2} DIAPHRAGM				Q_{O_2} LIVER				Q_{O_2} KIDNEY			
		Milligrams per kilogram	Number of doses	Number of days		Control	With addition	Change	Per cent change	Control	With addition	Change	Per cent change	Control	With addition	Change	Per cent change
Normal																	
1934																	
4/14	0				Sodium succinate	5.76	7.16	+1.40	+24	10.30	23.00	+12.70	+123	22.81	47.43	+24.62	+108
3/28	0				conc. M 0.018									26.22	47.80	+11.58	+82
3/17	24					5.00	7.15	+2.15	+43	6.00	29.00	+23.00	+386				
5/ 3	0				Sodium pyruvate	5.58	8.84	+3.26	+58	9.35	11.25	+1.90	+20	20.10	18.60	−1.50	−7
5/ 3	0				conc. M 0.018	5.60	8.24	+2.64	+47	10.90	15.02	+4.12	+38	21.12	21.08	−0.04	0
3/17	24					5.00	6.90	+1.90	+38	6.00	6.33	+0.33	+6	21.92			
4/16	0				Dihydroxyacetone					6.06	6.72	+0.66	+11				
4/14	0				conc. M 0.03									22.81	25.80	+2.99	+13
4/25	0					7.68	6.74	−0.94	−12					26.62	25.60	−1.02	−4
Hyperthyroid																	
3/27	0	5.0	9	11	Sodium succinate									32.55	57.75	+25.20	+77
5/ 7	24	10.0	3	4	conc. M 0.018	6.69	13.80	+7.11	+106	11.67	44.20	+32.53	+278	24.10	67.18	+43.08	+179
5/ 8	24	10.0	2	3		6.39	12.04	+5.65	+89	16.64	37.30	+20.66	+124	37.65	72.82	+35.17	+93
5/ 7	24	7.5	3	4	Sodium pyruvate conc. M 0.018	7.17	10.72	+3.55	+501	11.00	15.47	+4.47	+40	26.29	17.20	−9.09	−35
4/23	0	5.0	8	11	Dihydroxyacetone	10.87	9.00	−1.87	−17	13.88	15.83	+1.95	+14	28.65	29.58	+0.93	+3
4/21	0	5.0	7	10	conc. M 0.03	8.52	10.36	+1.84	+22	13.46	18.40	+4.94	+37	30.00	34.53	+4.53	+15
4/17	0	5.0	4	6		14.54	8.99	−5.55	−38	9.19	5.51	−3.68	−40	27.20	32.40	+5.20	+19

Experiments with succinate, pyruvate and dioxyacetone

In table XI are assembled the results of experiments in which sodium succinate, sodium pyruvate and dioxyacetone ($CH_2OH \cdot CO \cdot CH_2OH$) were added to normal and hyperthyroid tissues. The sodium succinate was prepared by neutralization of a solution of C.P. Succinic Acid Crystals with NaOH. Freshly distilled pyruvic acid, supplied by Professor E. K. Marshall, was neutralized with NaOH and the pyruvic acid determined by Wendel's (51) modification of Wieland's method (53). We are also indebted to Professor Marshall for supplying us with a sample of crystalline dioxyacetone.

The effects of pyruvate and of succinate were studied in order to determine the capacity of hyperthyroid tissues to oxidize substrates, other than lactate, that occur naturally in the animal body. Furthermore, it has been shown that muscle enzyme extracts from hyperthyroid animals reduce methylene blue in the presence of succinate more rapidly than do enzyme extracts of normal muscle (3). Dioxyacetone was employed because Mendel (30) has shown that it inhibits the respiration of Jensen sarcoma without affecting the Pasteur-Meyerhof reaction. Under similar circumstances inhibitors such as cyanide, carbon-monoxide, etc., cause great increases of aërobic glycolysis.

It will be seen from table XI that upon addition of succinate the rise in the Q_{O_2} of hyperthyroid diaphragm is 2 to 3 times greater than that of normal diaphragm. In the case of liver and kidney the variations are large but the hyperthyroid tissues seem to oxidize the succinate more rapidly than do normal tissues.

The addition of sodium pyruvate caused increases in the Q_{O_2} of hyperthyroid diaphragm and liver that were at least as large as those found in normal tissues. It is interesting that, in the case of kidney, addition of pyruvate actually caused decreases in the respiration of both normal and hyperthyroid tissues.

The action of dioxyacetone was variable both in normal and hyperthyroid tissues. In some instances it caused a considerable increase of respiration and in other instances a decrease. There was no constant inhibitory effect such as Mendel (30) noted in the Jensen-sarcoma.

DISCUSSION

During the past few years it has become increasingly evident that the fundamental changes in hyperthyroidism are the result of alterations in the metabolism of the tissue cells and that these alterations are not dependent upon an intact central nervous system. The earlier view that overactivity of the sympathetic nervous system was responsible for the metabolic phenomena of hyperthyroidism has gradually been undermined by accumulating data. It was first shown that neither muscular activity, muscular fibrillation nor increased muscle tonus could explain the increased metabolism of animals rendered hyperthyroid with thyroxine (5). Similarly, after transection of the cervical or thoracic spinal cord (20, 35), after removal of all sympathetic chains (42), after adrenalectomy (5) and after the cutting of all nerves to the extremities (41) the increased metabolism of hyperthyroid animals remains unaltered. The experiments reported in this paper and those cited from the literature demonstrate clearly that characteristic metabolic changes persist in the tissues after they have been completely isolated from the hyperthyroid animal. It would appear, indeed, from the experiments *in vitro* of Markowitz and Yater (28) and Paal (36) that thyroxine can act directly on the tissues without the necessity of having any other organ act as an intermediary. It is probable that all actively metabolising tissues share in the effects of thyroxine, the changes being dependent upon the type of metabolism normally present in the tissue. Cardiac and skeletal muscle, kidney, liver and nerve, alligator's red blood cells and tissue of a mouse's ear have all been shown to have an increased respiration following their removal from hyperthyroid animals.

The wide variations in the heightened respiration of different tissues from the same animal are not easy to explain. The average increases of respiration in the three tissues studied are of the same order when a large series of animals is considered, but in individual experiments the increase of respiration in one tissue is often much greater than that of the other tissues. Table I indicates that this has no relation to the time elapsing between administration of thyroxine and measurement of tissue respiration. The results in table I do, however, seem to indicate that the respiration of kidney and liver may be affected by a dose of thyroxine that affects muscle tissue but little.

It should be pointed out also that although large increases of tissue respiration can be demonstrated they are not as large as would be expected from the degree of increased oxygen consumption observed in the intact animal. Thus, even when the oxygen consumption of the animal is computed without regard to loss of weight, the mean increase of tissue respiration is only about 50 per cent as compared to 70 per cent for the intact animals. A further consideration that makes the divergence seem even wider is that a portion of the animal's weight is made up of fluid and of tissues, *e.g.*, non-nucleated red blood cells, bone, fibrous tissue, etc., that are probably affected to a lesser degree than the actively metabolising tissues that were studied.

Inasmuch as Dresel (11) performed his experiments in bicarbonate buffer and found increases up to 200 per cent in respiration of hyperthyroid liver tissue, it might be thought that bicarbonate buffer and an atmosphere containing 5 per cent CO_2 were necessary for the demonstration of maximal changes in tissue respiration. This point suggests itself because of the fact that complete inhibition of respiration by cyanides only occurs under such circumstances (2, 50, 10). Ebina (14), however, performed his experiments in bicarbonate buffer in an atmosphere of 5 per cent CO_2 and found no increases of tissue respiration comparable to those reported by Dresel.

The fact that the extra moiety of respiration due to hyperthyroidism is inhibited by cyanide is of considerable interest. According to modern conceptions (23) two main mechanisms are involved in normal cellular respiration. First, the enzymatic breakdown of various substrates by dehydrogenation (54) or dismutation (34, 15) reactions to simpler metabolites. This process occurs in the absence of oxygen and is little affected by cyanide. Second, the enzymatic activation of molecular oxygen by an oxidase and its passage over a reversibly oxidizable and reducible iron, containing pigment [cytochrome—Keilin (23); *Atmungsferment*—Warburg (37)] to unite with the activated hydrogen of the substrate. The second of these two mechanisms is inhibited by cyanide. Some observers (10, 45) believe that the sum total of normal cellular respiration may be due to a number of mechanisms of the second type, some of which are cyanide sensitive and others of which are cyanide resistant. It is clear from the experiments noted in table VI that the extra respiration due to hyperthyroidism is carried out by a mechanism that is cyanide sensitive.

Dye (12) has suggested that administration of thyroxine to an animal "leads to an increase in the oxidative capacity of muscle tissue, either by increasing the amount or potency of its tissue oxidases or probably by serving the rôle of co-enzyme or transportase." Dye and Waggener (13) have reported a decrease in the activity of indophenol oxidase in various tissues from thyroidectomized animals.

Following Haffner's (20) report, a number of workers (6, 14, 25) have attributed the metabolic changes of hyperthyroidism to an increase of anaërobic glycolysis in the tissues. Haffner suggested a primary involvement of the anaërobic phase of metabolism but did not specifically point to the anaërobic breakdown of carbohydrate. His hypothesis was based upon the following facts: (1) The long latent period required for the action of thyroxine. (2) The fact that accelerated metamorphosis of tadpoles due to thyroxine is not inhibited by cyanide while the lethal sensitivity to cyanide is greatly increased, the latter fact apparently due to interference with the increased oxygen needs of the organism. (3) The finding of a 50 per cent increase of lactic acid in tadpoles following several days of exposure to thyroxine.

Subsequent workers (6, 25) have used this hypothesis to explain the alterations in lactic acid metabolism that occur in the hyperthyroid organism. None of the evidence, however, provides definite proof that thyroxine exerts its primary action on the anaërobic glycolysis of the tissues and the facts are susceptible to other explanations. A full discussion of this point is given in another place (3).

In the experiments reported in this paper an increase of anaërobic glycolysis was found only in the case of hyperthyroid renal tissue. Liver and muscle tissue from hyperthyroid animals, although showing large increases in respiration, showed no increased capacity to form lactic acid under anaërobic conditions. Furthermore, tissues from hyperthyroid animals were no more sensitive to fluoride and moniodacetic acid than were normal tissues. These facts are strongly against the concept of a primarily increased glycolysis in hyperthyroidism. They have no bearing, of course, on the other phases of anaërobic metabolism. It is of interest, in this connection, that the respiratory quotient of hyperthyroid human beings or animals is usually somewhat lower than normal (48). It would seem important that studies

be made of the respiratory quotient of tissues from hyperthyroid animals.

It is possible that hyperthyroidism is associated with increased combustion of all the foodstuffs normally concerned in the metabolism of a given tissue. If it is true that about 50 per cent of the metabolism of kidney (8), 15 per cent of that of muscle and 50 to 100 per cent of that of liver (46) involves the combustion of carbohydrate, one would expect wide variations in the degree of increased respiration of different tissues if one type of foodstuff were alone involved in the metabolic changes of hyperthyroidism. The fact is that in a large series of experiments the mean increase of respiration is of much the same order in all the above mentioned tissues.

It might be assumed that the abnormalities of carbohydrate metabolism commonly observed in hyperthyroidism (3) are the result of a lowered power on the part of the tissues to oxidize or to resynthesize lactic acid. The experiments with lactate, pyruvate and succinate demonstrate that tissues from hyperthyroid animals are no less able than normal tissues to oxidize these substrates. Hyperthyroid muscle tissue, indeed, has an increased ability to oxidize them. Whether the normal ratio of oxidation to resynthesis (Meyerhof's *Oxydations-quotient*) exists in hyperthyroid tissues is a question not answered by the above experiments. It should also be pointed out that these experiments have been carried out under optimal conditions as regards oxygenation of the tissue and therefore show the inherent capacity of the tissue to perform its functions. It is conceivable that, in the intact hyperthyroid organism, the various mechanisms for transporting oxygen to the tissues are inadequate to meet the extra demands and that accelerated lactic acid formation or decreased lactic acid removal might appear as a secondary phenomenon.

In conclusion it is suggested that further studies of the increased tissue respiration due to hyperthyroidism might throw considerable light on the mechanisms involved in the respiration of normal cells.

SUMMARY

Using the Warburg method measurements have been made of the metabolism of skeletal muscle (diaphragm), hepatic and renal tissue from white rats following subcutaneous administration of thyroxine.

Control experiments were made in each instance with tissues from normal animals. At varying time intervals (22 hours to 8 days) following administration of a single dose of thyroxine (10.0 mgm. per kilogram) to the animals there was a small (up to 28 per cent) and variable increase in the respiration of hepatic and renal tissue. The respiration of muscle tissue underwent no significant change.

Induction of hyperthyroidism by multiple injections of thyroxine brought about large increases of tissue respiration. In 12 experiments the mean increase was 46 per cent for kidney, 73 per cent for liver and 75 per cent for muscle. In all succeeding experiments the animals were rendered hyperthyroid by a series of injections of thyroxine. In 7 experiments a comparison was made between the oxygen consumption of the intact animals and the respiration and anaërobic glycolysis of their isolated tissues. The mean increase of total oxygen consumption was 89 per cent and the mean increase of tissue respiration was 57 per cent for kidney, 45 per cent for liver and 53 per cent for muscle. The anaërobic glycolysis of liver and muscle tissue showed no change while that of kidney tissue was increased 91 per cent. Further glycolysis experiments carried out in M/700 cyanide confirmed the above results.

Methylene blue (conc. 0.005 per cent) caused similar changes in the respiration of both normal and hyperthyroid tisssues. The mean Q_{O_2} of renal tissue decreased 57 per cent in the normal series (8 experiments) and 62 per cent in the hyperthyroid series (7 experiments), the mean Q_{O_2} of hepatic tissue decreased 17 and 26 per cent in the two series, respectively, and the mean Q_{O_2} of muscle increased 38 and 29 per cent in the two series respectively. In the presence of methylene blue the anaërobic glycolysis of renal tissue was increased 172 per cent in the normal series and 50 per cent in the hyperthyroid series, the glycolysis of hepatic tissue was increased 106 and 36 per cent in the two series, respectively, and that of muscle tissue increased 12 and 22 per cent in the two series respectively.

The extra respiration due to hyperthyroidism was inhibited by cyanide (conc. M/70). Experiments with renal tissue in varying concentrations of cyanide showed that the extra respiration due to hyperthyroidism was inhibited by concentrations of cyanide that did not affect the respiration of normal cells.

The respiration of hyperthyroid renal and hepatic tissue was no more inhibited by fluoride and iodoacetate than that of normal tissue. Potassium iodide had no significant effect on the extra tissue respiration due to hyperthyroidism.

Aqueous enzyme extracts of muscle from hyperthyroid animals, when added to normal tissues failed to produce as large increases of respiration as did similar extracts from normal muscle.

As indicated by the increase of oxygen consumption upon addition of sodium d-lactate (M 0.018), hepatic and renal tissue from hyperthyroid animals had as great a capacity to oxidize this substrate as had normal tissues. Hyperthyroid muscle had a greatly increased capacity to oxidize lactate. Similar results were obtained upon addition of sodium succinate (M 0.018) and sodium pyruvate (M 0.018). Dioxyacetone (M 0.03) had no constant effect on the respiration of normal and hyperthyroid cells.

CONCLUSIONS

1. Representative tissues from hyperthyroid animals preserve an increased oxygen consumption after isolation from the animal body.

2. The increase in tissue respiration is only roughly proportional to the increase of total oxygen consumption by the intact hyperthyroid animal. The increase of tissue respiration is not as great as would be expected from the increase of metabolism in the intact animal.

3. The increased moiety of respiration in hyperthyroid tissues is not carried out by any new or abnormal respiratory mechanism in the cell.

4. An increase of tissue glycolysis is not the fundamental cause of the increased oxygen consumption in hyperthyroidism.

5. Cyanide, fluoride and moniodacetic acid reduce the respiration of hyperthyroid tissues but do not affect the fundamental mechanism which makes an increased supply of oxygen necessary to the organism. Iodine, when added directly to the tissue, has no specific effect on the increased tissue respiration due to hyperthyroidism.

6. Hepatic and renal tissues from hyperthyroid animals have no lessened capacity to oxidize various substrates (lactate, pyruvate, succinate). Hyperthyroid muscle has a greater capacity to oxidize these substrates than has normal muscle.

I wish to express my gratitude for helpful advice and criticism to Sir Henry Dale and Dr. J. H. Gaddum of the National Institute for Medical Research and to Dr. Edward P. Carter and Dr. E. Cowles Andrus of the Johns Hopkins Hospital.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

Clinical Science. By SIR THOMAS LEWIS. 189 pp. (*Messrs. Shaw & Sons, Ltd., London, 1934.*)

It seems almost axiomatic that a book by Sir Thomas Lewis must be interesting and stimulating. His ability to perceive where others merely look stands out in his writings. The chapters on auricular fibrillation and flutter are masterpieces of deductive reasoning; the one on arteriovenous anastomoses suggests the unlimited field presented to the clinician by experiment. What can be done with a simple observation by a thoughtful investigator is beautifully shown in the chapters on the active contraction of capillaries and the triple response of the skin to injury. The chapters on Reynaud's disease and Erythromelalgia are of monumental scepticism.

W. J. T.

Clinical Pathology of the Jaws. By KURT H. THOMA. 1934. 643 pp. $9.00. (*Charles C. Thomas, Springfield, Illinois, and Baltimore.*)

This is not just another book, but a real and complete post-graduate course, minutely detailed and explicit, thoroughly comprehensive and at the same time concise.

L. P. H.

A Textbook of Pathology. Edited by E. T. BELL. 2nd edition, thoroughly revised and enlarged. 767 pp. $8.50. (*Lea & Febiger, Philadelphia, 1934.*)

This edition has been greatly enlarged and has been revised by a group of five members of the medical and pathological staff of the University of Minnesota. They not only discuss the anatomical changes (with which most pathologists unfortunately content themselves), but also pay some attention to the physiological abnormalities underlying the disease. Unfortunately, these physiological considerations are too limited in scope and not always up-to-date.

A. G.

The Autonomic Nervous System. By ALBERT KUNTZ. 2nd edition, enlarged and thoroughly revised. Illust. 697 pp. $7.50. (*Lea & Febiger, Philadelphia, 1934.*)

This edition contains, in a well organized form, such a wealth of information concerning the autonomic nervous system that it offers an indispensable aid to

students of the subject. The author has been surprisingly successful in his effort to include the significant contributions of older as well as of more recent investigators. Nevertheless, the device of quoting conclusions of numerous workers without analyzing and evaluating their evidence leaves many controversial questions no clearer by virtue of such summaries. This, however, does not detract from the value of the volume as a reference book or from its usefulness to the experienced student.

Among other new features is to be found a discussion of the respiratory and circulatory functions of the carotid sinus, the chemical mediation of the autonomic nerve impulse, the reflex regulation of blood pressure and the autonomic representation in the corpus striatum and cerebral cortex. The sections devoted to the autonomic nervous system in disease and to its surgery have also been extensively revised and amplified.

C. McC. B.

Acute Intestinal Obstruction. By MONROE A. MCIVER. 430 pp. 62 Illust. $7.50. (*Paul B. Hoeber, Inc., New York, 1934.*)

This volume is, in effect, a second edition, the first having appeared serially in *The American Journal of Surgery.* Those familiar with the initial publication will recognize the competence in the present handling of the subject. The innumerable repetitions common to serial journal publication have been excised from the present edition.

The first part of the book considers in detail the types of intestinal obstructions and is by no means a mere catalogue of rarities. The second part which takes up diagnosis and treatment is excellent, but seems somewhat verbose and loosely put together. The last section which deals with the cause of death is not the unimaginative collection of indigestible experiments which the literature presents, but rather a carefully selected few analyzed for whatever they are worth.

W. J. T.

The Heroic Age of Science. By WILLIAM ARTHUR HEIDEL. 203 pp. $2.50. (*Williams & Wilkins, Baltimore, Md., 1932.*)

In spite of the very unjust criticism of Sarton, this survey of Greek science will commend itself to all interested in its development. The author is one of the earliest investigators of Greek medicine in this country, a veteran philologist, who has long since won his spurs. Dr. Welch, reading the volume, while in the hospital, defined it as a "soliloquy." In other words, it does not cover all the recent literature. But the author's intention is to present his own modest reading of the subject, which is clear, informing, understandable and preferable to a mere mechanical abstract of what others have said.

F. H. G.

4

NEUROMYELITIS OPTICA

An Anatomical-Pathological Study of One Case; Clinical Studies of Three Additional Cases

FRANK B. WALSH

From the Wilmer Ophthalmological Institute, Johns Hopkins Hospital

Submitted for publication January 24, 1935

Neuromyelitis optica is a term used to designate a neurological condition, the outstanding features of which are a rapid loss of vision and paraplegia. The predominant autopsy finding is demyelinization. This disease was first observed by Allbutt (1) in 1870 and was described by Seguin (2) in 1880. It was termed *neuromyélite optique aiguë* by Devic in 1894. This disease so closely resembles acute multiple sclerosis in both its clinical and pathological characteristics that there has been some debate whether it should be considered as a separate entity. It likewise somewhat resembles a disease described by Schilder (3) and termed by him *encephalitis periaxialis diffusa*, which presents sufficiently uniform pathological-anatomical characteristics, however, to allow its differentiation. The cause of these somewhat similar conditions, neuromyelitis optica, acute multiple sclerosis and encephalitis periaxialis diffusa, is unknown.

The cases described here appear clinically to belong to the neuromyelitis optica group for the following reasons: (1) Bilateral optic neuritis is rarely seen in multiple sclerosis, and as Brain (4) has stated, coincident bilateral optic neuritis with myelitis is unknown in multiple sclerosis. (2) Two of the cases here reported occurred in children less than 10 years of age, which is an unusual age incidence for multiple sclerosis. Wechsler (5) and Brain (6) have said that multiple sclerosis rarely occurs in childhood. Garrod and his associates (7) stated that it never occurs before 12 years of age. (3) The cases here reported were not self-limited as is the case in diffuse encephalomyelitis following infectious fevers and vaccination.

It is the purpose of this paper to report a clinical-pathological study

of one case of neuromyelitis optica and to report briefly three other cases which apparently belong to this disease entity.

Case I. (*History 79864. Path. No. 12822*)

On September 13, 1932 a white girl of nine was admitted to the Johns Hopkins Hospital with a diagnosis of tumor of the brain. She complained of blindness and inability to walk.

The family history was negative. Her birth and development were normal. During the summers of 1929, 1930 and 1931, she had had a skin affection described as "fall sores." These pustular lesions appeared early in the summer when she ran barefoot, and were limited to the legs below the knees and to the feet. They lasted three or four months in spite of treatment. She had uncomplicated measles in June 1931. Her tonsils and adenoids were removed in September 1931. She had never been vaccinated against smallpox. From her 6th to her 9th year she had "bilious attacks" with fever, nausea, vomiting and weakness. These usually lasted only a day or two and recurred two or three times a year. In 1932 she had two such attacks. One occurred in February and differed from previous attacks in that she complained of neuralgic pains starting in the thighs and radiating to the feet.

Present Illness: During February, March and April of 1932 she seemed to be nearsighted. On August 24, 1932, she had a bilious attack with yellowness of the skin and sclerae, but no itching. Two days later she appeared normal but for possible somnolence during one day, and for the following five or six days she seemed to be entirely well.

On August 31, 1932, her vision became affected and on the following day she was almost completely blind. Because of blindness she was put to bed. On September 5 she complained of her feet "going to sleep," and rubbing and massage were painful. She lost strength in her feet rapidly, first in the left, then in the right foot. During the first two days she could not stand alone but could move her feet. The paralysis then extended upwards to involve the legs and thighs. On September 11 she could sit up alone, but on the following day she was unable to do so. Control of the sphincters was lost. Sensory involvement was present at this time.

Examination: On admission she had a temperature of 100.2° F., a pulse of 88, and respirations of 40. Vision was limited to doubtful perception of light in each eye. The pupils were widely dilated and almost completely without reaction to light. The discs were slightly pale with blurred outlines on the nasal sides from low grade papilloedema.

There was moderate rigidity of the neck. She moved her legs and feet slightly, following painful stimulation. The paralysis was flaccid and almost complete. There was no contraction of the abdominal muscles when she attempted to sit up. There was almost total hypalgesia over the legs, buttocks, and lower abdomen, but temperature and touch were appreciated. Bone conduction was appar-

ently lost. The sense of position of the leg as a whole remained. The biceps and triceps jerks were normally active, whereas the knee and ankle jerks were sluggish but equal. Abdominal reflexes were not obtained. Hoffmann's, Babinski's, and Kernig's signs were absent. No ataxia was elicited.

The cerebrospinal fluid was under normal pressure, clear but contained flaky particles, the nature of which was not explained. There were 27 cells per cu.mm. of which 80 per cent were polymorphonuclears and 20 per cent were lymphocytes. The Queckenstedt test was negative. The spinal fluid was negative to culture. Chemical analysis showed 20 mgm. per cent non-protein nitrogen, 712 mgm. per cent chlorides, and 58 mgm. per cent sugar. The blood showed a 90 per cent hemoglobin and a white cell count of 12,000.

Since the admittance diagnosis of tumor of the brain was untenable, she was transferred from the neurosurgical to the pediatric service.

On examination at that time her temperature was 38°C., pulse 120 and respirations 28. She weighed 40 pounds, whereas the ideal weight for her age was 70 pounds. She was apathetic and lay on her side, with her head retracted and her spine arched in a position of semi-opisthotonos. She complained of pain in her neck. She was completely oriented for time, place, and persons, and seemed of good mentality. The skin was warm, moist, very loose, flushed in spots, and showed persistent dermatographia lasting for two to three hours after stimulation. Examination of the eyes revealed no change. The lips were cracked, the breath was foul and the tongue coated. The neck was rigid and movement of the head was extremely painful. Heart and lungs were normal. The abdomen was scaphoid. There was a flaccid paralysis of the legs. The arms could be moved voluntarily but were weak. The abdominal, knee and ankle reflexes were absent and the biceps and triceps jerks were sluggish. A suggestive Kernig's sign was elicited. There was incontinence of urine and faeces. The sense of pain, touch and temperature was absent below the nipple line, whereas above this line there was hyperaesthesia to pain.

Laboratory Data: WBC 11,360; RBC 4,400,000; Hbg. 85% (12.3 gms.). Differential: PMN 65.3%; PME 1.3%; PMB 1.3%; L.L. 4.7%; S.L. 24.7%; Monos. 2.7%. Smear: Normal.

Urine: albumin—trace, faint; diacetic acid—very faint trace; acetone—++; occasional WBC; granular and waxy casts present.

X-ray showed the cervical and thoracic vertebrae to be normal.

Wassermann negative.

Tuberculin (Mantoux): Negative to 0.1 and 1.0 mgm. O.T.

Blood culture: Repeatedly negative.

Vaginal smear: Negative for gram-negative diplococci.

Throat culture: Few colonies of Beta Streptococci. Many colonies of Alpha Streptococci.

September 17. Cerebrospinal fluid: Cloudy; Pandy +; Culture—negative; Pressure 85 mm. water; Wassermann—negative; Protein—50 mgm.%; Chlorides

—702 mgm. % as NaCl; Queckenstedt—normal; Cells—? 1300 per cu.mm. with 96% PMN; 2% lymphocytes. Colloidal Mastic Test—2,232,100.

September 17. Urine culture: Negative.

Course: The temperature ranged between 38° and 39°C. from September 15 to September 19 and then between 37° and 38°C. up to September 26. There was urinary retention with overflow incontinence. The patient had to be catheterized repeatedly until some five or six days before death, when the bladder became automatic. Dermatographia was marked throughout the entire course of the disease. At first it was present on the lower extremities, abdomen and chest, but later (by September 19) it extended upward to involve the neck and face where it remained most marked. At this date the paralysis had ascended above the nipple line. The level of touch and pain hypaesthesia extended higher up on the left than on the right side, *i.e.*, to the second interspace on the left and the fourth interspace on the right. The areas above were hyperaesthetic. The patient complained that her hands and arms "felt like they were going to sleep," just as the feet had felt at the onset of the illness. At this time a bilateral Kernig's sign was present. The paraesthesias persisted for four or five days and the strength and voluntary movements of the arms slowly decreased. On September 22 there was unilateral hyperhydrosis and flushing of the right side of the face sharply demarcated by the midline. The level of tactile anaesthesia extended still higher. The sense of position of fingers, hands and arms was gone and the sense of pain and touch over both ulnar regions was very poor and practically absent. The process showed signs of upward progression. By September 24 the senses of touch and pain were still further involved and more of both the flexor and extensor surfaces of the arms were anaesthetic. There was, however, some difference between the two sides, the right being more involved. The anaesthetic areas were irregular in distribution and somewhat spotty. Hot and cold discrimination was also affected over the same areas. On September 20 another lumbar puncture was done and a fairly clear, somewhat yellowish fluid under a pressure of 35 mm. water was obtained. Pandy's test showed increased globulin. There was no tryptophane in the fluid. The cell count was 37 per cu.mm. The total protein was 419 mgm. per cent. On September 24 a similar fluid was obtained. On this day the patient seemed more apathetic than previously. She was drowsy and complained of "pins and needles" sensations about the mouth and on the tip of the nose. She could not talk as loudly or as clearly as before. The facial muscles, as well as those of the tongue, moved well. Sense of touch was completely absent over both arms and up to the clavicles on the thorax, though strong painful stimuli were felt at times as evidenced by a weak cry. There was complete flaccid paralysis of arms and complete loss of sense of position. There was anaesthesia of the face. She grew progressively weaker and after September 26 cooperation was very poor, and apathy and drowsiness were so great that sensory examinations were unreliable. A catheterized specimen of urine showed a positive culture of B. coli communis. It was very cloudy, showed 50 to 75 W.B.C. per highpower field in the uncentri-

fuged specimen, and contained a trace of acetone and diacetic acid. After September 28 her temperature was constantly elevated varying between 38 and 41°C. Further neurological examinations could not be made because of the extreme weakness, apathy and drowsiness of the patient. During the first week of October the intercostal muscles ceased to function, the diaphragm alone maintaining respiration. The pallor of the optic discs was increased. She began to have difficulty in swallowing. Speech became slurred and indistinct. Her nose, hands and feet became cyanotic and slightly swollen. She was unable to swallow for several days before her death on October 16.

Case I. Autopsy 12822 (Dr. John H. Biggert)

Only the examination of the brain and cord has been detailed from the complete autopsy record.

Cranial cavity: The meninges were thin, the dura being adherent to the calvarium. The venous sinuses were patent.

The brain was covered by a smooth, shining pia mater. The blood vessels were not distended. The cranial nerves showed no gross lesions. The retinae were somewhat pale. The bony sinuses were normal.

The spinal meninges were thin, and the extradural tissues normal. At the level of the 5th thoracic segment the blood vessels of the cone became congested, and the cord itself became discolored. The bluish-black discoloration due to haemorrhage was marked throughout the lower thoracic and upper lumbar regions. In the lumbar region the cord was extremely soft, and on section was semi-fluid in consistence. Section of the cord at the level of the 5th thoracic segment showed some injection of the anterior horns, and some yellow, translucent areas in the posterior and lateral columns. Sections in the cervical region appeared relatively normal, though the white matter was generally rather more translucent than normal.[1]

Following fixation, the brain was cut in slices one centimeter thick. The brain seemed to be of normal consistence. In the frontal areas immediately beneath the cortex of the second frontal convolutions were several small, oval areas somewhat more translucent than the surrounding white matter. Many such areas were scattered throughout the corona radiata, becoming more numerous and larger as the occipital pole was approached. These scattered areas varied in size from small flecks which seemed to be around blood vessels to large areas 1 to 2 cm. in diameter. The larger areas were often stippled with yellowish flecks. Practically the whole of the white matter around the calcarine fissure gave the impression of being altered. None of these changes were seen to involve the cortex.

Similar flecks of translucent tissue were present around the red nucleus. The medulla appeared normal. The cerebellum showed no lesions. Further section

[1] The left half of the brain, excepting the cerebellum, was studied in the Phipps Psychiatric Clinic.

of the cord after fixation revealed the presence of translucent areas in the posterior and lateral columns in the cervical and upper thoracic segments. At the level of the vith thoracic segment softening commenced. This gradually extended in area until in the xth thoracic segment the whole diameter of the cord was involved and its anatomic characteristics were lost. This continued to the termination of the cord. In places the softening was so extreme that there was cavitation.

Brain: Sections were stained with haematoxylin and eosin, Mallory's phosphotungstic acid haemotoxylin, Van Gieson's connective tissue stain, Weigert's stain for myelin sheaths, Loyez's stain for myelin, Foot's stain for axis cylinders, and Nissl's stain. Frozen sections were stained with Scharlach R for fat.

The H. and E. stains showed that the yellowish, translucent areas observed macroscopically were areas in which there had been destruction of nerve tissue, the only tissue remaining being the glial framework. The cell content of such areas was increased. These areas were entirely subcortical and faded gradually into the surrounding tissue. There was no perivascular infiltration.

While there were such scattered areas throughout the subcortex, the process was most marked in the occipital lobe, especially in the region of the calcarine fissure (fig. 6). Small areas were present in the basal ganglia and neighborhood of the red nucleus.

Weigert's myelin stain showed that the essential lesion in these areas was a demyelinization. Small perivascular lesions were also revealed by this stain in the midbrain and around the larger areas in the occipital lobe (fig. 12).

Glial stains failed to show the presence of any gliosis. The astrocytes were swollen, and in some cases possessed two nuclei, but such cells were very infrequent. Axis cylinder stains showed that there was no destruction of the axis cylinders in the centre of the larger areas, but towards the periphery of each axone the axis cylinders seemed to be less affected than the myelin sheaths.

Fat stains showed that the affected areas were full of fat which was mainly intracellular in compound granular corpuscles (fig. 11). These were present in large numbers and showed some tendency to aggregate about blood vessels (fig. 13).

Nissl granule stains showed that the cortical and basal ganglion nerve cells were apparently normal. The cells of the midbrain showed no lesions.

Cerebellum: No lesions were found.

Optic Nerve: The H. and E. stain showed a slight increase in the number of cells. The blood vessels were distended (fig. 3). At the level of the chiasm fat stains showed a dense infiltration of fat-laden cells, especially marked around blood vessels. Weigert stains at this level showed complete demyelinization (fig. 4).

(Sections of the posterior half of each eye showed only a slight papilloedema. Weigert stain showed slight demyelinization close to the globe, increasing toward the chiasma (figs. 1 and 3). There was a focus of demyelinization in the tract (fig. 5).—F. B. W.)

Spinal Cord and Medulla: At the bifurcation of the pyramidal tract the pos-

terior half of the cone showed rarefaction. Smaller areas of rarefaction were present in the anterior half, but these were irregular in position and outline, and the process was evidently not a system degeneration. Myelin sheath stains showed that practically the only fibres escaping were the motor tracts.

As one progressed down the cord the lesion gained in severity. In the cervical region the whole cord, with the exception of a few fibres around the periphery, showed a loss of myelin (fig. 7). In the upper thoracic segments a few fibres still remained relatively intact around the cord margin, but even there showed swelling and distortion of the myelin sheath (fig. 9). As one descended, small areas gradually appeared in which there was destruction of even the glial framework and nothing was left except a collection of fat-containing cells. In the viiith thoracic segment there were macroscopic cavities in the posterior columns containing a few compound granular corpuscles (fig. 10). The whole lumbar cord was disorganized and consisted of necrotic débris of fat-containing macrophages.

There was no gliosis at any level. Axis cylinder stains showed that in the cervical thoracic regions these were somewhat less involved than the myelin sheaths, but caudally they were completely destroyed.

The anterior horn cells in the cervical and upper thoracic regions surprisingly showed no lesions. At lower levels there was chromatolysis and disintegration. The efferent and afferent nerves showed little change. Demyelinization, if present, was more marked in the posterior roots. Many of the posterior roots showed a central core of myelin destruction which terminated abruptly as the nerve left the cord. It was not until the lumbar segments with their complete disintegration were reached that the demyelinization in the nerve roots became marked.

In interesting contrast to the extreme changes in the cord is the fact that the pons and upper medulla showed no lesions. The process, therefore, did not seem to have produced any obvious ascending or descending tract degeneration.

Peripheral Nerves: The brachial plexus, sacral plexus, obturator nerves, and intercostals were examined. These showed little change, a few scattered fibres in the sacral plexus showing fatty degeneration of their sheaths.

Anatomical Diagnosis: Multiple foci of demyelinization in brain with involvement of optic nerve. Extensive demyelinization of the spinal cord. (Acute multiple sclerosis? Schilder's disease? Neuromyelitis optica?). Acute haemorrhagic cystitis, pyelitis, pyelonephritis. Terminal pneumonia.

SUMMARY OF CASE I

A white child of 9 years died six weeks after the onset of an acute disease process attacking the brain and cord, resulting in blindness and ascending motor and sensory paralysis. Of particular interest, in that it proves the remitting nature of the affection, is the history of an attack of pain in the limbs and visual disturbances some three

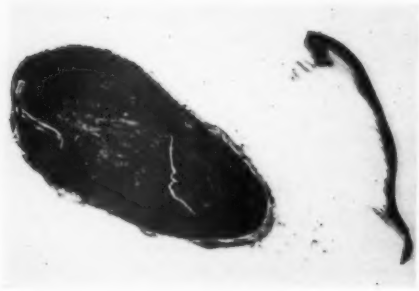

FIG. 1. SECTION OF RIGHT OPTIC NERVE CLOSE TO THE GLOBE (WEIGERT STAIN)
Slight evidence of demyelinization

FIG. 2. OPTIC NERVE CLOSE TO THE CHIASMA
More extensive demyelinization than in preceding figure

FIG. 3. SLIGHT INCREASE IN CELLS OF OPTIC NERVE

FIG. 4. COMPLETE DEMYELINIZATION OF CHIASMA (LEFT HALF)
Several incompletely demyelinized areas are apparent (Spielmeyer)

FIG. 5. AREA OF DEMYELINIZATION IN LEFT OPTIC TRACT (SPIELMEYER)

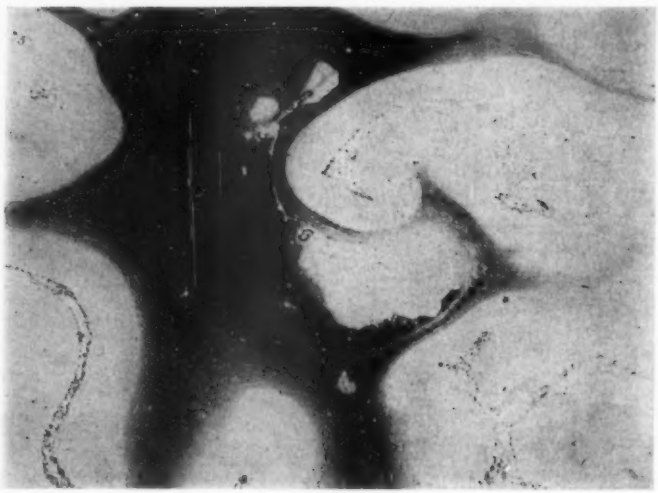

FIG. 6. DEMYELINIZED AREAS IN THE OCCIPITAL LOBE

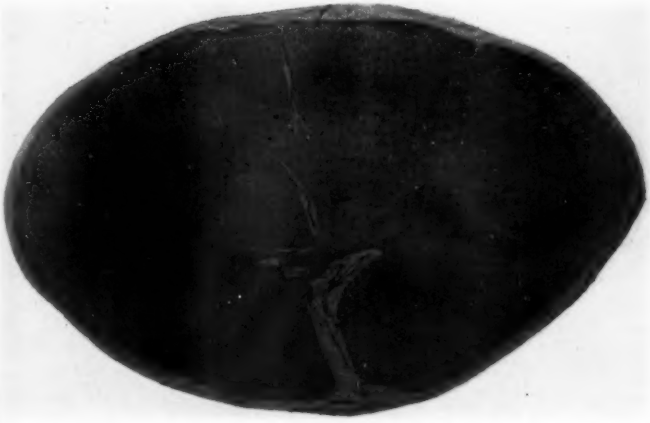

FIG. 7. INVOLVEMENT OF THE CERVICAL CORD

FIG. 8. THORACIC CORD (WEIGERT STAIN) AREAS OF DEMYELINIZATION

FIG. 9. SECTION AT LEVEL OF 8TH THORACIC SEGMENT, SHOWING MACROSCOPIC CAVITATION

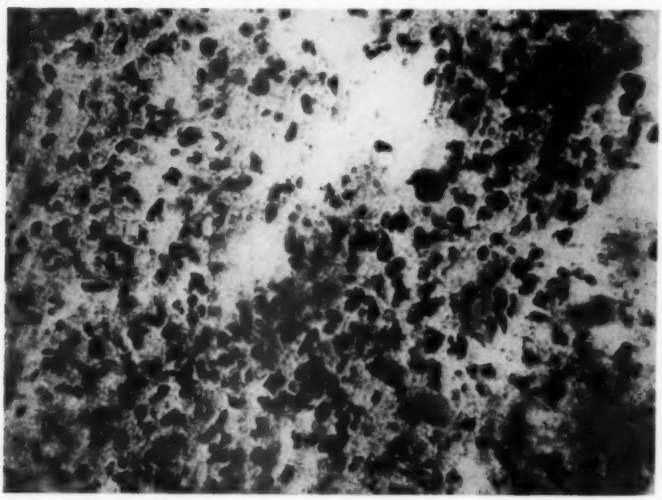

FIG. 10. COMPOUND GRANULAR CELLS CONTAINING FAT
Section from occipital lobe

FIG. 11. AREA OF PERIVASCULAR DEMYELINIZATION IN OCCIPITAL LOBE (SPIELMEYER)

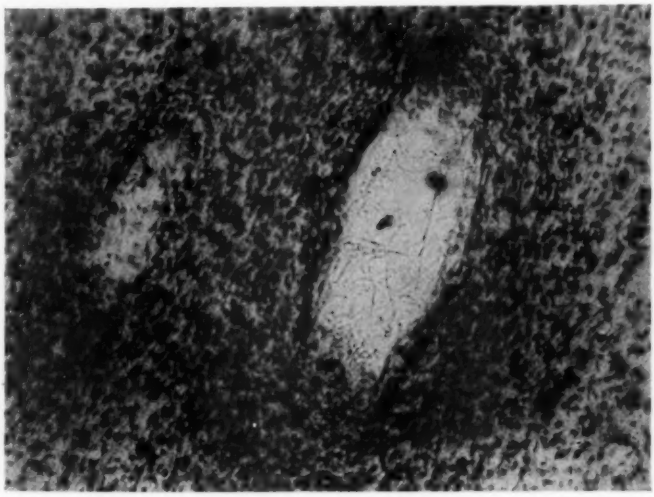

FIG. 12. SECTION SHOWING FAT-FILLED CELLS AGGREGATED ABOUT BLOOD VESSELS

months prior to the onset of the fatal illness. An ascending urinary infection and terminal pneumonia were secondary findings.

Small areas of translucent appearance were seen throughout the brain, well confined to the white matter. Practically all the white matter around the calcarine fissure appeared altered. The cerebellum was unaffected, the basal ganglia practically unaltered. The cord was much more extensively affected. The cervical region appeared relatively normal. At the level of the vth thoracic segment the cord became discolored a bluish black from haemorrhage, and lower in the lumbar region the cord was soft and semi-fluid.

Microscopic study with the appropriate stains showed intense demyelinization, an absence of gliosis, a minimum of perivascular infiltration, and some sparing of axis cylinder.

The optic nerveheads were slightly pale but sections at this level showed only a slight papilloedema. Posterior to the globe the optic nerves showed partial demyelinization and increased cellularity (figs. 1, 2, and 3). There was complete demyelinization at the chiasm, slight involvement of the optic tract, and extensive destruction of white matter in the occipital lobe (figs. 4, 5, and 6).

The following two cases were observed clinically and in the light of the case just reported appear to belong to the neuromyelitis optica group. The first case is included only because of the early age incidence. The second differs from the reported case in the manner of onset.

Case II. (*History 74271*)

D. B., a white girl 9 years of age, was admitted July 10, 1931, with marked dysarthria and a spastic paralysis of the legs. The family and past history was non-contributory.

In February 1931 she had a mild upper respiratory infection diagnosed as influenza. In the middle of March 1931, after recovery from this attack, she developed cramp-like pains in the calves of the legs. These gradually grew worse and in the second week of April she began to drag her left leg. After three days her left ankle gave way and she fell, when she was sent to bed by her physician. On the following day she lost rectal and vesical control. The left leg became still weaker and spastic. At this time the tentative diagnosis of neuritis was changed to one of infantile paralysis. She was sent to Sydenham Hospital and remained about five months. For the first three days of this time she ran a temperature.

The right leg became weak and spastic. There was a complete sensory loss to the level of the nipple line. During her stay there she suffered from headaches and occasional nausea.

After she returned to her home, sensation gradually returned from above downwards and she regained rectal and some vesical control. Three weeks before her admission to the Johns Hopkins Hospital she developed dysarthria which became steadily worse.

On admission her temperature was normal. She appeared mentally normal, but emotionally unstable. General examination was negative but for the presence of several small opened vesicles, in an area as large as the palm of a hand, over the left inguinal region. There was moderate generalized kyphosis, most marked in the lower thoracic region. Neurological examination showed sluggish jaw jerks, feeble forward and lateral movements of the mandible, relaxation of right angle of mouth and lower right face. There was marked dysarthria, but no hoarseness, regurgitation or difficulty in swallowing. The tongue could not be wholly protruded and deviated slightly to the right. There was no atrophy or tremor. The spinal muscles appeared weak. The upper extremities were normal. The abdomen was soft. Both legs were spastic, particularly the left. There was moderate paresis in all leg movements. No sensory level could be ascertained.

Orientation and intelligence were normal. There were no cerebellar signs.

The abdominal reflexes were absent; the patellar and Achilles reflexes were hyperactive. The Babinski sign on the left was positive but indefinite on the right.

After two weeks in the hospital she was discharged to her home.

On September 25, 1931 she was markedly improved. The dysarthria had disappeared so that her speech was almost normal. The motor power of the right leg had improved but the left was but slightly better and showed contractures. She was free of sphincter disturbances. She had started to go to school in January 1932 and was able to climb two flights of stairs several times daily.

On April 26, 1932, after having complained of pain about the right eye for a week with increasing visual loss, she was admitted to the Wilmer Institute. Her vision at this date was R.E.V. $\frac{1}{200}$, L.E.V. 20/15. The right eye showed a papilloedema of 3 to 4 diopters and a large absolute central scotoma. The supranuclear type of facial palsy on the right persisted; otherwise the cranial nerves were normal. Movements of the left hand had become somewhat clumsy and there was questionable intention tremor. The legs were weak and spastic, particularly the left. The abdomen bulged in the flanks when she lifted her head. The right extensors of the spine were weak. The gait was spastic and unsteady. Speech was clear. There was no loss of deep or superficial sensibility, no astereognosis, no loss of position or vibration sense. There were no areas of anaesthesia or paraesthesia. The tendon reflexes were increased in the arms; knee jerks were increased, the right more than the left; the left ankle jerk was absent, the right

active; the abdominal reflexes were present but feeble and easily exhausted. There was a positive Babinski sign bilaterally. The cerebro-spinal fluid showed 1 cell per cu.mm., Pandy reaction negative, sugar present.

A diagnosis of neuromyelitis optica was made, which was concurred in by the neurologists. She was discharged May 3, 1932 with R.E.V. 5/200, L.E.V. 20/15.

On December 28, 1933 the vision in each eye was 20/15 uncorrected. Examination of the fundus was negative and one could not say there was definite pallor of the right disc. She showed difficulty in walking, both legs still being rather spastic, and she still complained of wetting the bed at night.

Case III. (*U-16395*)

On January 13, 1928, H. G., a white girl of 17, was first seen in the Ophthalmological Dispensary complaining of marked loss of vision in each eye.

Ten weeks before she had noticed that the vision in the right eye was defective. This loss of vision was marked and had not been accompanied by other symptoms. About January 1, 1928 she noted dimness of vision in the left eye which increased rapidly.

The family history was unessential.

She had been taken out of school when she reached the seventh grade because of extreme nervousness. She suffered from occasional attacks of abdominal pain, a severe one having occurred eighteen months before the onset of eye symptoms. She had occasional attacks of sore throat, but none immediately preceding the loss of vision.

Examination of the eyes showed a slight anisocoria, the right pupil being somewhat larger than the left. The pupils reacted to light and convergence. There was no ptosis nor nystagmus. There was definite pallor of the disc on the right and questionable pallor of the left disc. The margins of the nerve in each eye were clear cut. R.E.V. 3/200. L.E.V. 6/200.

General physical examination at this time showed a dental sepsis, white cells in the urine, and a slightly elevated blood pressure. Nephritis and hypertension were later ruled out. The usual laboratory tests, including a blood Wassermann, were normal.

She was admitted to the hospital on January 21, 1928. At this time the left eye was completely blind. The right eye showed a contracted field with colors much sharper nasally. A diagnosis of pituitary tumor was made, and on January 24 a left craniotomy was performed. The left optic nerve was severed during the operation, this eye being blind. An intracranial tumor was not present. She made an uninterrupted recovery from the operation.

On February 10, 1928 she developed a transient ankle clonus. Polyuria and polydypsia of mild degree were present. The vision in the right eye increased to 15/100. She was discharged on February 11, 1928.

In May 1928 she developed a numbness and weakness of the right leg which lasted for a month. In April 1930, when four months pregnant, she complained of

soreness and numbness of both legs, sufficient to make her go to bed for one month. During June she got about on crutches.

In November 1930 she began to lose vision in the right eye and after four days was completely blind. She reported to the dispensary December 5, 1930, at which time she was completely blind. The pupils were dilated, with no reaction to light. There was advanced bilateral optic atrophy. Examination at this time showed the other cranial nerves to be quite normal. The abdominal reflexes were absent. The tendon reflexes were slightly more active than usual. There was no clonus, no Babinski. The motor system was negative. There was no loss of sphincter control. The sensory system showed no change. The spinal fluid showed no cells and a negative Wassermann.

The vision improved and by January 20, 1931, she could count fingers, and early in May the vision had improved to 10/200. She was discharged to her home, her case being definitely diagnosed as one of multiple sclerosis. This diagnosis was later changed to neuromyelitis optica because of the completeness of the visual loss, and subsequent development of transverse myelitis. She was able to do her housework and did not again attend the hospital until 1933.

There had been occasional frontal headaches ever since the operation, frequent colds and attacks of tonsillitis.

On February 1, 1933 she complained of dull aching pain in the epigastrium and under the sternum, with some involvement of the arms. The legs had pins and needles sensations, the left leg being the first affected. On February 3 she was unable to void. The following day the legs were completely paralyzed. The neck became stiff and sore. There was no vomiting, no loss of consciousness, and no further visual disturbance.

On February 6, 1933 she was readmitted to the hospital. The temperature was 100, pulse 104, respirations 19, blood pressure 130/100. She was well nourished and did not appear in acute distress. There was marked distention of the bladder. Neurological examination showed the cranial nerves normal with the exception of the second. The left pupil was dilated and fixed to light. It reacted, consensually. There was marked optic atrophy, more marked in the left eye, and great attenuation of the vessels in each eye. The motor system showed paralysis of the abdominal muscles and spastic paralysis of both lower extremities. There was no motor weakness of the upper extremities. There was a definite sensory level at T.III, below which all forms of sensation were lost or modified. The vibratory sense was absent on both sides below T.VI The position sense of the lower extremities was absent There were no cerebellar or cerebral signs. The abdominal reflexes, knee and ankle jerks were absent The Babinski was positive bilaterally There was no clonus or Kernig sign.

Laboratory tests showed W.B.C. 11,000, Hbg. 80%, a mere trace of albumin and sugar in the urine, no red cells or casts. The spinal fluid showed 120 mm. of water pressure, 34 cells, 80% polymorphonuclear, the remainder lymphocytes.

There was a positive Pandy reaction, negative Wassermann, negative mastic curve. The spinal fluid showed N.P.N. 21 mgm.% and sugar 20 mgm.%.

A Horner's syndrome developed on the left side, from involvement of the cervical sympathetic. By February 15 improvement had set in, both as regards muscle power and sensation, but on February 22 she developed a cystitis and ureteritis. Several lumbar punctures had been done, all similar to the one on admission. By this date the motor system had improved yet further and the sensory system was better. The reflexes were but slightly changed. By March 4 still further improvement was evident and some control of the bladder had returned. On April 1 a lumbar puncture showed only 2 cells per cu.mm. On April 2 she was discharged to her home improved.

She was readmitted May 2, 1933. The cranial nerves, excepting the second, were as before—quite normal. The spasticity in the legs remained. There were still present extensive sensory changes, absent abdominal reflexes. The Babinski was questionably normal on the right but showed dorsal flexion on the left. She was still having difficulty in voiding.

She was discharged improved with R.E.V. 15/100 on August 27, 1933. The ptosis had disappeared.

SUMMARY OF CASE III

A girl of 17 had been diagnosed finally as suffering from neuromyelitis optica. She presented as a first symptom marked loss of vision in both eyes, the left becoming completely blind. Because of field changes and bilateral visual loss, a diagnosis of pituitary tumor was made. This was proved to be in error by exploratory operation. Paraesthesias and motor weaknesses made their appearance. For about two years she was able to get about. Recurrence of eye symptoms appeared and she became completely blind in her useful eye, but again recovered vision. About three years later she had a most severe attack with paraplegia and paraesthesias, from which she partially recovered.

A summary of a fourth case history is added. It is quite different from those already described.

Case IV. (K-11741)

M. S., a white woman of 29, was first seen in the Ophthalmological Dispensary on February 4, 1933.

She gave a history of typhoid fever in June 1921. After apparent recovery she had a "nervous" spell in August 1921, with sharp, stabbing pains in the face, diplopia, and an internal strabismus. These symptoms disappeared in a few weeks. In August 1922 she complained of severe pains starting at the base of

the skull and radiating up over the right side of the head. The vision of the left eye decreased to total blindness within a few days and after an interval of weeks; the right eye was similarly affected. In January 1923 she developed a flaccid paralysis of the left side. In February 1923 sensation and motor function returned; soon after vision improved to perception of light in both eyes. The condition remained unchanged until August 1932 when she complained of a smoky sensation in front of the eyes. She had visual hallucinations on two occasions. For several months prior to examination she complained of pain in the right side of the face.

At the time of examination the vision was reduced to perception of light in each eye. The right eye showed a divergent concomitant strabismus of 25°. There was a slow horizontal nystagmus with the quick component to the left. The nerveheads were dead white in color, the margins sharply outlined, and the arteries slightly attenuated.

Neurological examination revealed no abnormality of the other cranial nerves. There was no definite motor weakness. There was a suggestion of a Babinski sign on the left. The abdominal reflexes were active on the right and present in the left lower, but tired easily. The only sensory abnormality was a decrease of the vibratory sense in the legs, more marked on the left.

Laboratory examinations were negative. These included complete examinations of the blood and spinal fluid.

Up to the present date her condition has remained unchanged.

Of particular interest are (1) the occurrence of trigeminal pain, (2) nystagmus, (3) external strabismus that had been preceded by a paralytic convergent squint, and (4) visual hallucinations. The onset, however, was unlike multiple sclerosis and accordingly this case is included as one of neuromyelitis optica.

COMMENT

Nomenclature: It is yet to be determined whether such cases are an unusual manifestation of acute multiple sclerosis or acute disseminated encephalomyelitis. They are clinically unlike multiple sclerosis, and are not self-limited as is disseminated encephalomyelitis. In consequence, they can be considered clinically either as a definite disease entity, or as a sub-group of multiple sclerosis or disseminated encephalomyelitis. Terms such as myelitis with optic neuritis, ophthalmoneuromyelitis or neuromyelitis optica have some descriptive value and we have used this last term.

Goulden (8) outlined the views of early observers who believed the optic nerve involvement to be a secondary manifestation of the central nervous system disturbance, due either to increased intracranial pressure, an ascending meningitis, or to vasomotor paralysis through

the sympathetic system. It is now generally agreed, however, that a common agent, whatever it may be, is responsible for all the symptoms, and that the optic nerve symptoms are an integral part of the disease process.

Loss of Vision: This is usually the first symptom in a majority of cases, although there is not general agreement on this point. Beck (9) found that the majority exhibited paraplegia as the first symptom but Goulden's (8) figures indicated that 80 per cent of these cases commenced with loss of vision as the primary symptom. Three of our four patients showed loss of vision as the initial sign. Both eyes are usually affected, rarely simultaneously, the interval ranging from a few hours to several days or weeks. Case II was an exception in that only one eye was attacked. Lapersonne (10) has reported such a case. The loss of vision is rapid. In a few days, or even a few hours as recently reported by Chang (11), there may remain mere perception of light, or there may be complete blindness. The vision tends to improve within weeks or months, and may occasionally become quite normal, as in the case reported by Perritt (12). Pain in or about the eye preceding loss of vision is complained of in a minority of cases.

Muscle Palsies: Extra-ocular muscle palsies are not usual. Cases III and IV are exceptions to this generalization. Case III exhibited a transient ptosis due to involvement of the cervical sympathetic and Case IV a paralytic squint with diplopia followed by concomitant external strabismus.

Pupils and Pupillary Reactions: These are not distinctive. The degree of dilatation of the pupil usually corresponds to the amount of visual loss in the eye.

Cortical or cerebral blindness has not been described in this condition as far as I am aware. In the closely related disease, *encephalitis periaxialis diffusa* (Schilder's disease), cortical blindness, when present, has been considered a diagnostic sign of great importance.

Ophthalmoscopic Appearance: There may be a wide variation in the appearance of the fundus oculi. Examination may reveal an apparently normal fundus in a completely blind eye. On the service of Dr. Adolf Meyer there occurred such a case in which the chiasma was found to be completely demyelinized. One completely blind eye showed a normal fundus until death occurred five weeks after the

vision was first affected. In Case II here reported there was an oedema at the disc of between 3 and 4 diopters, the veins were engorged and haemorrhages and exudates were present. The appearance may simulate a papilloedema from intracranial pressure due to tumor, but there is a marked early loss of vision that is not a feature of choked disc. Klar (13) has described a case similar to Case II and explained the oedema by assuming that the focus was in the nerve close to the disc. In Case II the visual field of the affected eye showed a central scotoma and there was a history of pain in and about the eye prior to the loss of vision. In consequence this should be considered as a type of retrobulbar neuritis with papilloedema as described by Rönne (14).

These two extremes are not usual. Most frequently early cases show slight blurring of the margins of the disc and some degree of venous dilatation. Pallor of the whole disc rather than of the papillomacular bundle is frequently seen later in severe cases. This pallor may increase until the disc is entirely white. With this change there is marked attenuation of the retinal vessels.

Fields: As the areas of demyelinization are irregularly arranged, and the chiasma frequently involved, it is reasonable that many types of field change are described.

Central defects are the most frequent. A suggestion of hemianopsia in Case III led to an erroneous diagnosis of brain tumor. Erb (13) was the first to report such a field defect in this condition. Ward Holden (16, 17), who reported 5 such cases, considered this particular field defect to be of great diagnostic importance. Klar (13) saw homonymous hemianopsia for colors. He also described quadrantic defects with crescent-shaped extensions. Central scotomata, particularly for colors, are frequently seen, and concentric constriction may be found. A central scotoma may remain when the peripheral field has widened, as in a case recently reported by Chang (11).

Nystagmus: This is infrequent. Case IV offers an exception.

Paraplegia: This may precede or follow the visual symptoms by days or months; or may appear simultaneously. Beck (9) stated that, although fluctuation in vision was usual, the paraplegia usually progressed until either death occurred or improvement began. He considered his case as unique, in that the paraplegic symptoms abated

at times. Certainly Case III exhibited waves of paraplegia. The sphincters are usually involved.

The legs are usually affected, first one and then the other. There is loss of sensation usually extending to the nipple line, above which there may be a zone of hyperaesthesia. There may be either signs of a transverse myelitis, a diffuse myelitis, or an ascending myelitis. The reflexes may be completely lost or exaggerated. Guillain and associates (18) have described a case characterized by epileptiform jerkings in which the shoulders were first affected.

Prognosis: The intensity of the disease varies within wide limits. Death may occur in a few days (Marc-Adrien Dollfus (19) and Chisholm (20)), or an almost complete recovery may take place in two or three months or longer. Various observers estimate the recoveries at about 50 per cent. Brain (4) stated that the tendency in disseminated sclerosis for more complete recovery in the remission is a differentiating point from neuromyelitis optica, which leaves more residual loss.

Relation to Syphilis: Syphilis is not a factor in the great majority of cases, although several are reported in which there is a definite history of syphilis. Cobbledick (21) reported such a case. Milian Lhermitte and collaborators (22) reported a case of this nature with pathological studies and plates very similar to those of Case I. Schaeffer (23) suggested that lues might have been the causative factor in a case of neuromyelitis optica.

Confusion with Brain Tumor: Two of our cases were at one time or another erroneously diagnosed as tumor of the brain.

The extremely rapid loss of vision suffered by these patients is not seen with brain tumor but is characteristic of neuromyelitis optica, and may occur in acute disseminated encephalomyelitis or acute multiple sclerosis.

In neuromyelitis optica papilloedema is less marked than that frequently observed in brain tumor. In the latter type (choked disc) there is relatively slight loss of vision and the fields tend to be concentrically contracted rather than to show the central defects so common in neuromyelitis optica.

The inability to obtain an accurate history, the development of an optic atrophy prior to the development of the characteristic para

plegia, and the suggestive visual fields often found, may lead to an erroneous diagnosis of brain tumor, and subsequent craniotomy. Exploratory craniotomy or lumbar puncture in this general group of diseases may produce an exacerbation of the process or a meningeal reaction of such severity that death may result within forty-eight hours. Two such cases have been observed recently. Exploratory operation in one instance and lumbar puncture in the other accounted for an early demise. The autopsy diagnosis was multiple sclerosis.

Acute Multiple Sclerosis: These cases here reported differ clinically from multiple sclerosis in (1) the extreme youth of the patients (Cases I and II); (2) the occurrence of bilateral optic neuritis with myelitis (Cases I, III, and IV); and (3) the occurrence of bilateral blindness (Cases I, III, and IV) of far greater severity than that in multiple sclerosis, where it is unusual for an eye to become totally blind suddenly, and bilateral blindness is even rarer.

The pathological differences (Case I) are (1) absence of involvement of the cerebellum; (2) the presence of cavitation in the cord; (3) widespread continuous demyelinization in the cord; (4) absence of gliosis.

Beck (9) pointed out similar differences in his case in 1927. Symonds (24) and McAlpine (25) have considered such changes to be merely a manifestation of a particularly acute type of multiple sclerosis. Case II might be considered as one of multiple sclerosis but for the age of the patient.

Encephalomyelitis Following Infectious Fevers and Vaccination: These conditions are self-limited. Sager and Grigorescu (26), who made a direct comparison, pointed out that ophthalmoneuromyelitis (neuromyelitis optica) has a longer course.

Encephalitis Periaxialis Diffusa (Schilder's disease): In the past, cases now recognized as Schilder's disease were included in the group of diffuse sclerosis. Schilder's disease usually occurs in early life, although cases are reported as occurring in adults,

There is frequently bilateral blindness of rapid onset, sometimes of cerebral type, early mental deterioration, bilateral spastic paralysis and death in coma. The disease may last only a few days or several years. There is an extensive involvement of white matter. It has been described as beginning in the occipital lobes, accounting for blindness with retention of pupillary reflexes (cerebral blindness);

extending forward to the temporal lobe, accounting for central deafness, to the internal capsule, causing bilateral spastic paralysis; and finally reaching the frontal lobes, when there is mental deterioration. The cord is rarely affected. Ford and Bumstead (27) showed that the lesion may first appear in any part of the white matter. Stewart, Greenfield and Blandy (28) reported a case with involvement of the cord.

When fully developed this disease is not likely to be confused with neuromyelitis optica. Pathologically, it is somewhat similar but the cord is rarely involved, whereas in neuromyelitis optica it is extensively affected. The outstanding ocular sign in Schilder's disease is cerebral blindness However, this symptom was not observed in two patients in the Johns Hopkins Hospital in whom the diagnosis of Schilder's disease was confirmed at autopsy (U-11,897. Autopsy 9830) (U-19,806 Autopsy 10,500). Collier and Greenfield (29) have reported a case in which the fundus appeared normal and the pupils remained active until death occurred after 15 months. The same authors pointed out that no case of central scotoma from involvement of the extreme occipital pole had been reported up to 1924. They have said that when papilloedema was present it was of low degree, but in one of the autopsied cases above mentioned there was an oedema of 3 to 4 diopters with markedly increased intracranial pressure and death from respiratory paralysis.

Vision and fields are invariably affected. Bouman (30), in his review, stated that choked disc occurred in a minority of cases and temporal atrophy of the disc was an exception. Many cases showed normal pupillary reactions, some showed sluggish reactions, while some failed to react. Disturbances of the ocular muscles were found in less than half the cases, usually in the form of strabismus, diplopia and disturbance of conjugate movement.

Hemianopsia may be observed first from one side and then from the other, resulting in complete blindness. Altitudinal hemianopsia has been reported.

Gasul (31) has reported a case and reviewed the literature up to 1930.

Diffuse Sclerosis: Conditions of even rarer occurrence than Schild-

er's disease make up this group. These affections are familial, occur at an early age, and are progressive. The three principal types are briefly described.

(A) Pelizaeus-Merzbacher's disease: Pelizaeus described a progressive type of involvement of the nervous system in a family later described further by Merzbacher (32). Batten and Wilkinson (33), in reporting 4 cases, gave the following clinical description: "The disease begins in the first months of life; it makes rapid progress until the sixth year, then it is more slowly progressive. When the disease is fully developed, it shows itself by nystagmus, slow speech, difficulty in carrying out motor impulses, disturbances of succession and coordination of movements, ataxia, intention tremor, *Mitbewegungen*, masque-like facial expression, paresis of the back, pelvic and abdominal muscles and spastic contraction of the lower extremities, increase of the patellar reflexes, Babinski response, loss of abdominal reflexes. The following symptoms often accompany the above: trophic disturbances of the bones, vasomotor disturbances in the lower extremities, loss of mental capacity. The affected individuals may reach old age and only die of some intercurrent disease." It occurs usually in males. The three cases described by Batten and Wilkinson (33) showed a "wheel nystagmus."

(B) Krabbe's Familial Early Brain Sclerosis: This is an infantile form in which children apparently healthy at birth develop fits of crying and screaming at 4 to 6 months of age. There is a rapid, downhill course evidenced by failure of intelligence, epileptiform convulsions, visual failure, nystagmus, squint, and progressive spasticity of the limbs with paroxysmal intensifications, and finally a state similar to experimental decerebrate rigidity with difficult deglutition and death due to a terminal bronchopneumonia.

Nystagmus was present in all of Krabbe's (34) cases, internal squint in two cases. Optic atrophy appeared late. In one case optic neuritis was observed.

(C) Scholz's Juvenile Type of Diffuse Familial Cerebral Sclerosis: Scholz (35) has reported a condition similar to that described by Krabbe (34), occurring in children from 3 to 8 years of age. His cases showed cranial nerve lesions; ocular palsies, anisocoria, loss of pupil-

lary reflex, loss of corneal reflex, facial weakness, difficult articulation. There was mental deterioration, progressive visual failure, spastic paralysis of the extremities, loss of sphincter control, and cerebellar ataxia. All had a fatal termination.

Ophthalmoscopic examination in some instances was negative and in others showed temporal pallor.

SUMMARY

(1) A clinical-anatomical study of a case of neuromyelitis optica is recorded. Three clinically similar cases are more briefly detailed.

(2) Although these and similar cases may be a manifestation of an acute type of multiple sclerosis or of diffuse encephalomyelitis, they are sufficiently alike to warrant their designation by a special title.

(3) Errors in diagnosis may occur in distinguishing this affection from tumor of the brain. A precise knowledge of the sequence of symptoms should eliminate this possibility.

(4) This apparent clinical entity may readily be confused with other relatively rare neurological conditions.

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URGENCY AND FREQUENCY OF MICTURITION IN NEUROLOGICAL DISEASE[1]

ORTHELLO R. LANGWORTHY AND LLOYD G. LEWIS

From the Sub-Department of Neurology and the James Buchanan Brady Urological Institute, the Johns Hopkins University and Hospital

Submitted for publication February 4, 1935

INTRODUCTION

The behavior of the urinary bladder has been studied in patients with neurological lesions who complain of urgency and frequency. The bladder capacity and residual urine were determined. Cystometric readings were obtained and the intravesical pressure read as the bladder was distended with an increasing quantity of fluid. These cystometric readings were characteristic, making it possible to define this type of abnormality from other bladder lesions. The appearance of the bladder wall was observed with the cystoscope.

The patients in these tests were suffering from disseminated sclerosis, and we found it possible to correlate roughly vesical symptoms with lesions involving different pathways in the nervous system. Individuals affected with disseminated sclerosis are troubled most often by urgency and frequency which may partially incapacitate them for years.

We were particularly interested in the cystometric readings described here, inasmuch as similar deviations from normal had previously been produced experimentally. Langworthy and Kolb (1933) observed a markedly decreased bladder capacity after decerebration. They concluded that tone in the smooth muscle of the urinary bladder was controlled by a mechanism located in the mid-brain, and that this control could be demonstrated after cerebral influence was removed. These conclusions were supported by further experiments in which it was shown that stimulation of localized areas near the aqueduct of

[1] This study of the control of the bladder by the central nervous system was supported by a grant from the National Research Council.

Sylvius in the mid-brain caused the intravesical pressure to rise (Langworthy and Kolb, 1935). The same experiments showed the influence of the cortex in the control and initiation of micturition. The cortico-spinal tract appeared to carry the impulses concerned in bladder control from the cerebral cortex.

METHODS

The cystometric readings were carried out with an open water manometer made of glass tubing of three-millimeter bore. All portions of the apparatus which made contact with the fluid circuit to the bladder of the patient could be disconnected for sterilization. An air trap separated the aseptic from the recording portion of the apparatus. The pressure changes were read by observing the changes in height of water in two arms of a U tube. An air vent made it possible to equalize the pressure on the two sides of the U tube when the readings were begun. The whole apparatus could be raised or lowered so that the base line corresponded with the level of the bladder. The readings were recorded in centimeters by means of a sliding scale. Sterile oxycyanide of mercury solution was allowed to flow slowly into the bladder from a gravity bottle which was raised only a short distance above the patient. After each 50 cc. had entered the bladder the flow was interrupted and a reading made when the pressure had reached a resting level. We believe that a water manometer has certain advantages for experimental work over the one filled with mercury which is sold for diagnostic use. The former is extremely labile in its response and is inexpensive, easy to build and operate. With this instrument it is possible to record the small waves of contraction in an irritable bladder. Also, from time to time records of straining pressure can be obtained when the patient is asked to make a strong effort to empty the bladder. The importance of this procedure was suggested by Watkins (1934). Even so the water manometer used here was not as sensitive as the one we have employed in experimental work with animals (Langworthy, Reeves and Tauber, 1934). This is due to the difficulty of arranging an apparatus that can be sterilized.

We believe that it is possible to distinguish a true contraction of the bladder musculature from a rise of intra-abdominal pressure associated with contraction of the abdominal wall. A true bladder con-

traction is recorded by the manometer as a slow, sustained rise of pressure, often accompanied by the escape of fluid around the catheter, whereas a rise of pressure due to contraction of the abdominal wall is quick, unsustained, and never accompanied by escape of fluid. Of course in certain cases contraction of the abdominal wall may set up a secondary contraction of the bladder musculature.

EXPERIMENTAL

1. A cystometric reading upon a normal individual

There is still considerable doubt as to what constitutes a normal cystometric curve. For this reason we will discuss a reading upon an individual with complete insight into the problem and present his observations while the bladder was being filled; the graphic record is shown in figure 1. This man had never suffered from any disturbances of bladder control and his bladder was considered perfectly normal. The record of equal additions of fluid to the bladder volume is placed on the ordinates, and the intravesical pressures in centimeters of water on the abscissae.

After the catheter was passed, the subject felt slight but continuous discomfort which was always localized in the anterior urethra. The pressure readings were remarkably low until the bladder was completely filled and the impulse to void was irresistible. During all this time the subject felt that he was making a conscious effort to relax the bladder.

The desire to void came first when the bladder held 200 c.c. of fluid and was experienced as an increase in the sensation at the external meatus. After 250 c.c. had run into the bladder the desire to micturate was present all the time. After 350 c.c. had been introduced he experienced an unpleasant feeling in the perineum as if he were using the muscles to put off micturition. The desire to void was always increased as the water was slowly added. After 400 c.c. had entered the bladder and the pressure had fallen to 1.5 cm. of water, there was an urge to void. This desire was still present when the pressure was 1 cm. of water. It was suggested that the subject relax and cease voluntary control. He was able to do this and felt more comfortable. When 450 c.c. had been added, the pressure fell to a level between 4.5 cm. and 2.5 cm. At one time a rise of pressure oc-

curred to 10 cm.; it then began to fall again. With 500 c.c. of fluid in the bladder the micturition reflex could no longer be controlled. The pressure fell to 4 cm., and then slowly rose to 27 cm. with the escape

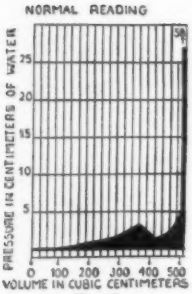

Fig. 1

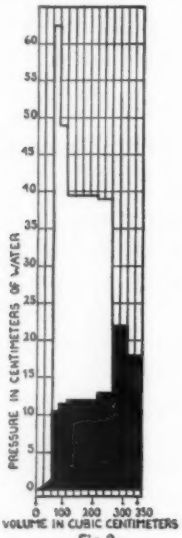

Fig. 2

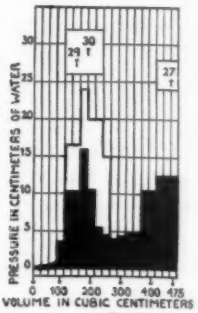

Fig. 3

FIG. 1. The addition of equal quantities of fluid to the bladder volume is plotted on the ordinates, and the intravesical pressure in centimeters of water on the abscissae. This reading of bladder pressure after the addition of equal volumes of fluid was made upon a normal individual who had never complained of symptoms referable to the bladder.

FIG. 2. This reading was made upon a patient who complained of urgency and frequency of micturition (case 1). The upper edge of the black area indicates the lowest pressure recorded after the addition of equal quantities of fluid; the white area indicates the range of vesical contractions.

FIG. 3. A reading of intravesical pressures on the introduction of equal quantities of fluid in case 2. The white area again indicates the range of bladder contraction during the early stages of filling.

of fluid around the catheter. There was a characterisitic chilly sensation with the onset of micturition. When the subject was asked to strain, the pressure rose to 58 cm. of water.

What findings characterize this curve as that of a normal individual?

Certainly not the pressure readings during the period of filling for they were extremely low. Indeed they would be perfectly characteristic for a patient suffering from bladder disturbances related to tabes dorsalis. We attribute the low readings in this case to the conscious effort of the subject to relax and restrain bladder contractions. A notch in the curve (fig. 1) occurred when the bladder was filled to 400 c.c. The pressure, which had been 3.5 to 2.0 cm. on 350 c.c. of fluid, fell at this point to 1.5 cm., indicating in all probability the result of a conscious effort at relaxation.

The most important feature of this normal curve is undoubtedly the inability to control the micturition reflex when 500 c.c. of fluid had entered the bladder. The pressure then rose and fluid escaped from the urethra around the catheter. Patients with bladder abnormalities due to defective nerve supply become uncomfortable with distention of the viscus, but no sustained contraction of the wall initiates emptying, even though the intravesical pressure slowly rises. The uncontrolled micturition at the end of filling is the most important feature of a normal bladder.

As the normal bladder is being filled with fluid, few slow rises and falls of pressure are seen aside from the respiratory waves. Unsustained contractions appearing frequently during slow distention are characteristic of a bladder with damaged innervation. This will be discussed more fully in connection with the examples which follow.

2. A study of the bladder function in two patients who complained of urgency and frequency

A number of patients have been examined with similar results. The findings will be discussed in two of these.

Case 1. D. K. The patient was an unmarried white man, 28 years old. Ten years before, while in college, he had had an illness which was called a nervous breakdown. At first his left leg had become weak and dragged; somewhat later the trouble had spread to his right leg and arm. He was in a hospital for three weeks and had almost recovered in three months, although his legs have always been somewhat weak since that time. Every spring or early summer he has been liable to an acute relapse of the symptoms.

He was first seen in this hospital three years ago during one of these acute attacks. He complained that the left leg gave way on walking, and the right leg was weak. Examination revealed a horizontal nystagmus on looking to either

side. At times there was a rhythmical tremor of the head. The left arm was weak; the deep reflexes were hyperactive in this arm and there was a positive Hoffmann test. The abdominal and cremasteric reflexes were difficult to obtain and tired easily. The gait was unsteady but there was no Romberg sign. The heel-knee test brought out slight ataxia, more marked on the left. The deep reflexes were hyperactive in both legs. There was bilateral ankle clonus and a Babinski reflex on the left. No sensory disturbances were demonstrated. The blood and the spinal fluid Wassermann were negative.

The patient has not had an attack of weakness of the legs for 2 years, and now feels better than in the last 10 years. During these 2 years the deep reflexes in the legs have remained hyperactive but it has not been possible to demonstrate an ankle clonus or Babinski reflex. The nystagmus and head tremor can still be demonstrated and the hands show an intention tremor when he is tired or excited. There is a slurring of a word or two in his speech under the same conditions.

At about the age of 10 the patient believes that he first noticed urgency and some frequency. By 15 the urgency was as great as it is now but the frequency was not so severe. The trouble with his legs did not begin until he was 18. He always has to leave a moving picture at least twice. Recently he had to micturate eight times during an hour after drinking a milk shake. Frequency and urgency are more severe toward evening. He has to get up once during the night. If he does not empty the bladder when the desire comes on, involuntary voiding often occurs. Sometimes the bladder holds a large quantity. After drinking beer or any alcoholic beverage he does not have much trouble, but micturition is frequent when it once begins. For the last four years there has been a loss of potency, although libido is still present.

Two cystometric readings were made upon this patient, only the second of which was entirely satisfactory. There was marked spasm of the external sphincter so that it was necessary to introduce the soft catheter on a stilet. With 50 c.c. of fluid in the bladder there were active, wide oscillations of pressure from 30 to 62 cm. of water. With 100 c.c. the pressure varied from 17 to 46 cm. of water. The catheter was forcefully ejected from the bladder after 150 c.c. had been introduced.

The second cystometric reading made two weeks later was more informative, inasmuch as the catheter was held in the urethra when the pressure was high; the results are shown in figure 2. This type of chart is not entirely satisfactory for recording the findings. The black area indicates the lowest pressures recorded after the introduction of additional equal amounts of fluid and the light area the extent of pressure variations seen in the manometer as waves. The pressure remained low until 75 c.c. of water had been introduced. It then showed oscillations between 67 and 10.5 cm. As further fluid was introduced into the bladder, the base pressure gradually rose while the height of the waves of oscillation decreased.

The intensity of the discomfort associated with filling did not vary directly

with the height of the pressure. The urgency and discomfort were always great whenever a marked wave of bladder contraction was evident. With 250 c.c. in the bladder the patient was comfortable when the pressure was 17 cm. A moment later with the pressure at 16 cm. he became very much distressed, and said he wanted to micturate badly. With 350 c.c. of fluid in the bladder the pressure remained fairly constant between 19 and 17 cm. of water without any marked fluctuations, but the patient felt considerable pain so that the fluid was allowed to escape by disconnecting the catheter from the manometer.

The effect of psychic stimuli was marked during the early stages of filling when the bladder was irritable; at this time the opening or closing of the door or entrance of someone into the room would initiate a wave of contraction in the bladder musculature. On the other hand, talking quietly to the patient and urging him to relax caused the pressure to fall when it was high.

The unusual findings in this cystometric reading are the marked irritability of the bladder musculature, the high pressures in the early stages of filling, the fall of pressure and loss of waves of contraction as the bladder became distended, and the inability to initiate micturition around the catheter when the urge to micturition became great. Also the severe pain associated with distention occurred even when the bladder pressure was relatively low.

After the first cystometric examination the interior of the bladder was examined with the cystoscope. There was considerable trabeculation with hypertrophy of the detrusor musculature. The trigone appeared elevated. The interureteric ridge was elevated, the ureteral orifices were normal. There was neither evidence of obstruction at the vesical orifice nor relaxation of the internal sphincter.

Cystometric examinations caused the patient temporary distress in the form of tenesmus and pain on micturition. Two weeks after the first examination he had better control of the bladder than he had had for years.

Case 2. A. G. This case shows the bladder abnormalities at the beginning of an acute exacerbation of the disease. A white married man of 37 had noticed weakness and numbness of the right leg for a year. Five months ago he began to have pain in the lower portion of the back, tingling in the right foot, decreased libido and impotence. For two months there had been a right-sided headache, weakness of the left leg, urinary urgency, frequency and occasional incontinence. Constipation became a marked symptom, and on one occasion there was fecal incontinence.

Examination showed signs that related mainly to the legs. The patient had a characteristic, cheerful, optimistic disposition. The cranial nerves were normal. There was slight ataxia of the arms especially on the left. Tone of the musculature of the arms was normal, but the deep reflexes were hyperactive and there was a bilateral Hoffmann test. The abdominal and cremasteric reflexes were absent. The legs were weak and somewhat spastic. There was a bilateral Babinski sign. The blood and the spinal fluid Wassermann were negative. The mastic test was normal. A Queckenstadt test revealed no block.

The first cystometric reading was made on the day when the patient was obliged to stop work because of weakness of the legs (fig. 3). After the introduction of 150 c.c. of fluid, the bladder musculature became hyperirritable and the pressure readings varied from 10 to 18 cm.: there was a desire to empty the bladder but no pain. At 175 c.c. the pressures were from 16 to 24 cm. and from 10.5 to 20 cm. with 200 c.c. in the bladder. The patient felt quite comfortable when the pressure was 10.5 cm. The waves were much less marked when the viscus was distended with a greater quantity of fluid; the pressure remained between 4 and 5 cm. until the bladder held 375 c.c. With the introduction of more fluid the pressure gradually rose so that it was 12.5 cm. when the experiment was terminated with a bladder volume of 475 c.c. With this amount of fluid in the bladder the patient was able to raise the pressure on straining to only 27 cm., and was unable to micturate around the catheter.

This reading again shows irritability and high intravesical pressures during an early stage of filling, and the inability to express fluid around the catheter when the bladder was full.

This patient also illustrated the effect of voluntary control of the irritable bladder. At the beginning of the experiment with the intravesical pressure high, the pressure fell when the patient was urged to relax and postpone micturition. As long as he kept his mind on this control the pressure remained low, but it rose at once if his attention was diverted from the bladder by getting him to talk upon some unrelated subject.

The symptoms in this case became rapidly more severe so that he was not able to walk and could scarcely move the legs and at the same time urinary incontinence became more frequent. At this time a cystometric reading showed an extremely low pressure and almost total absence of waves during the early stages of filling. The pressure was 3 cm. after 400 c.c. had been introduced. At a greater volume small waves of contraction appeared. The bladder could be filled to 1000 c.c. without discomfort. At that point the resting pressure was between 21 and 26 cm., and he could raise it to only 28 cm. by effort.

This patient was then cystoscoped. The external sphincter had an increased tone. The trigone was a little elevated, and there was trabeculation of the bladder wall. The interureteric ridge was raised. Fluid flowed from the bladder slowly, and there was lack of expulsive force.

DISCUSSION

The symptoms of urgency and frequency in the cases we have studied appear to be related to damage of the lateral columns of the spinal cord in the neighborhood of the cortico-spinal tracts. The first of the patients had experienced periods when signs of cortico-spinal tract damage were severe, but the bladder symptoms had been present longer than the attacks of weakness in the legs and persisted

when the only abnormalities demonstrable in the lower extremities were hyperactive deep reflexes. The second patient had more obvious signs of pyramidal tract injury.

In both these patients there remained some voluntary control of the smooth muscle contractions of the bladder wall. It seems possible that this control was mediated from the cerebral motor cortex along intact fibers of the cortico-spinal pathway or it may have acted upon the tonic mechanism in the mid-brain. One patient lowered the intravesical pressure on voluntary relaxation, but it rose suddenly when the attention was distracted. The other could also lower the intravesical pressure when he attempted to relax; the pressure rose if his mind was diverted by conversation. The voluntary control was faulty, allowing the pressure to rise to great height in the early stages of bladder filling, and maximal concentration was required to induce relaxation. The difficulty of lowering the pressure produced urgency which necessitated micturition to avoid incontinence. The frequency is in itself a function of the urgency. Inasmuch as the pressure rose with only a small amount of fluid in the bladder, the wall became adapted to a small size. It is possible that this contracture in itself gave rise to the appearance of trabeculation, without any real increase in the musculature of the bladder wall. However, that the irritability may produce enough increased work to cause true muscle hypertrophy seems to be the case.

If the urgency and accompanying frequency of micturition are dependent on the loss of normal cortical control of lower centers, it must be assumed that the cortical effect upon the smooth muscle of the bladder wall is essentially one of inhibition. However, we do not agree with Denny-Brown and Robertson (1933, B) that the only supra-spinal factor in the function of micturition is in the cerebral cortex. Our experiments have demonstrated a mid-brain mechanism presiding over tone in the muscle of the bladder wall. Indeed the cases under discussion recall the experiments of Langworthy and Kolb (1933) and show an overaction of the tonic mechanism in the mid-brain after normal cortical control has been removed.

The fibers which convey impulses from the mid-brain also run in the lateral columns of the spinal cord in the neighborhood of the pyramidal tracts. In the first patient we believe that these fibers are

intact whereas the cortico-spinal fibers are damaged. Both these groups of fibers were eventually rendered functionless in the second patient with the development of incontinence, and loss of marked waves of contraction during the early stages of filling. If the pyramidal fibers were functionless without damage to the fibers from the mid-brain, incontinence would have occurred at frequent intervals. This condition is seen in patients with bilateral lesions in the internal capsule.

Should the urgency and frequency be considered irritative or release phenomena? The history of the presence of these symptoms for thirteen years in the first patient makes it clear that they must be dependent on release from cortical control. None of the patients who were examined had evidence of cystitis which might make the bladder hyperirritable. These patients escape cystitis because they usually empty the bladder completely. When the wall was stretched until it was no longer subject to the spontaneous waves, the ability to produce a marked voluntary contraction of the bladder was lost. These individuals must rely upon the strong waves of contraction during the early stages of filling in order to empty the bladder. If the viscus became distended beyond the point at which this irritability was active, acute retention might easily result.

In a consideration of lesions of the central nervous system it is impossible to speak of the sympathetic or parasympathetic portions of the autonomic system. There is here a control of the function of micturition as a whole. Denny-Brown and Robertson (1933, B) believe, on the other hand, that the cortical control is finally effective through the medium of the parasympathetic fibers. However, the bladder of small capacity which must be emptied frequently is similar to the type produced experimentally by cutting the sympathetic fibers to the bladder (Langworthy, Reeves and Tauber, 1934).

It is important to emphasize the voluntary control of the smooth muscle of the bladder wall which was so well demonstrated by Denny-Brown and Robertson (1933, A). The only possible voluntary control through the sphincters consists of further closure of the external sphincter. The spincters relax when the intravesical pressure is raised by contraction of the bladder wall. In describing neurological lesions we should speak not of faulty sphincter control but rather of damaged innervation of the bladder musculature.

After making a record of bladder pressures during filling, little information can be obtained from a knowledge of the pressure at which the viscus accommodates equal additions of fluid. Low pressures may mean either a loss of tone in the muscle or an ability of the patient to relax completely. In experimental work a record of pressure during filling is more important, inasmuch as a normal reading can always be made before the experiment is begun and the pressures can be read with the animals anaesthetized. Waves of contraction occurring frequently during filling suggest a damaged innervation. A normal individual will raise the intravesical pressure and empty the bladder around the catheter, when the volume of fluid becomes great enough to render him no longer able to restrain micturition.

Pain associated with bladder filling is dependent on actual distention of the bladder wall and is less closely related to the intravesical pressure. Thus an individual may feel great distention at one pressure and a moment later be comfortable with the pressure several centimeters higher. The large waves of contraction are of course associated with a strong desire to empty the bladder. The normal individual felt marked distention when the pressure was only 1.5 cm. of water.

Stretching of the bladder wall by fluid is the adequate stimulus that produces the waves of contraction; in the cases described here the stretch reflex is hyperactive. It may be assumed that this hyperactivity of the stretch reflex is similar to that observed in the striated musculature after lesions of the cortico-spinal tract. In the normal bladder micturition is dependent upon a fusion of the waves of contraction produced by stretching into a tonic contraction giving rise to emptying of the bladder. In the present cases there was also fusion of the waves so that the pressure remained high for considerable periods. After the bladder was further distended with fluid, the stretch reflex became less active.

An explanation may be given of the way in which the results reported here differ from the experimental findings after similar lesions reported by one of us in *Brain* (1933). In the experimental animals the stretch reflex also became active during the early stages of filling. The animals were anaesthetized and the bladder emptied itself at this point. There were certain cases in which the pressure rose but emptying did not occur. With further filling the pressure tended to rise slowly, but the waves subsided. At this time which was early in the

bladder experiments, we attributed the results to faulty technique. In the work with humans the contraction waves were observed during the early stages of filling, but every effort was made to control them and to postpone micturition. The catheter was held in place to prevent its extrusion by the bladder contractions. It was also found that the patients had some control over the contraction by voluntary effort, and this was utilized to prevent micturition. The result was that the patients could be carried over the stage of urgency, and bladder filling continued.

It is difficult to devise a method for giving these patients relief from their symptoms. Certainly no improvement would be expected from cutting either the parasympathetic or sympathetic nerve fibers to the bladder wall. Section of the parasympathetics would serve only to impair further the already damaged function while, from a theoretical viewpoint, section of the sympathetics would tend to maintain the bladder in its small size. Stretching of the bladder wall by the introduction of fluid after the method described in this paper may give at least temporary relief; this is suggested by our studies. The immediate result is to increase the symptoms due to the irritation, but after this early discomfort was gone, two of our patients reported improvement. This method should not be used for treatment unless it is clear that the neurological lesion has been stationary for a considerable length of time.

SUMMARY

A study has been made of the symptoms of bladder urgency and frequency in patients with disease of the central nervous system. Records were obtained of intravesical pressure during bladder filling, and the interior of the viscus was examined with the cystoscope. Marked waves of contraction occurred when the bladder contained small quantities of fluid, but these waves tended to decrease and disappear as distention was continued. The patients showed some ability to reduce the contraction waves by voluntary effort. After the bladder was well filled, the patient had little power to increase the intravesical pressure voluntarily. These patients had partial lesions of the cortico-spinal pathway, and it was concluded that there was an overaction of a tonic mechanism in the mid-brain after cerebral control was partially removed.

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A STATISTICAL SURVEY OF 186 CASES OF MANUAL REMOVAL OF THE PLACENTA

C. H. PECKHAM, M.D.

From the Department of Obstetrics, the Johns Hopkins University and Hospital

Submitted for publication February 5, 1935

The literature of the last twenty years contains a number of articles dealing with the operation of manual removal of the placenta. Although the figures reported vary considerably, the authors are in general agreement that the procedure is dangerous, with a high incidence of puerperal sepsis and maternal deaths. Bumm, in 1909, put the gross mortality following this operation at 10 per cent. Three years later, Alletsee wrote a thesis on the subject and noted that 81 per cent of his assembled cases were in multiparae, and that in 10 per cent there was a history of manual removal of the placenta following a previous delivery. At this time, Rogoff collected mortality figures from a number of European clinics which varied from 2 to 13.9 per cent. In 1918, Hirst found the incidence of the operation to be 1 in 312 deliveries and stated that a 7 per cent death rate from sepsis followed it. Bourne, in 1921, reported a 35 per cent incidence of puerperal sepsis in his cases. In the same year, Baumm published the results from the Breslau Frauenklinik showing a 34.7 per cent morbidity and a 28 per cent mortality. In 1923, Goethals published an excellent review of the literature, together with his figures on the subject. For his own series of 170 patients, he reported an incidence of 2.07 per cent in hospital cases, 25 per cent sepsis, and a gross mortality of 8.2 per cent, with a corrected rate of 1.76. Montag in 1926 found that manual removal had been necessary in 0.97 per cent of 15,100 cases at the Bochum Clinic in Germany, with a mortality of 4.76 per cent, and a morbidity rate of 30.6 per cent. Finally, in 1928, Heidler and Steinhardt analysed 1,000 cases of manual removal at the Würzburg Clinic, with 82 deaths. The following table shows the causes of death in their series and it will be seen that 10 deaths due to sepsis occurred among the 483 patients on whom

manual removal of the placenta was the only operation done, a sepsis mortality rate of 2.07 per cent.

Deaths in 1,000 cases of manual removal of the placenta (Heidler and Steinhardt)

	MANUAL REMOVAL ALONE	MANUAL REMOVAL PLUS OTHER OPERATION	TOTAL CASES
Hemorrhage	6	22	28
Sepsis	10	18	28
Other causes	3	23	26
Tuberculosis	2	1	3
Cardiac disease	1	5	6
Eclampsia	0	8	8
Ruptured uterus	0	5	5
Anaesthesia	0	2	2
Diabetes	0	1	1
Paralytic ileus	0	1	1

Moreover, the above authors found a morbidity rate of 29.52 per cent for their series, excluding those patients that died, and noted that the puerperium was febrile in 25 per cent of those women whose only operation had been manual removal as contrasted with 34.14 per cent when delivery necessitated some other operative procedure.

From the foregoing brief analysis of the recent literature it will be seen that the problem of manual removal of the placenta has been adequately considered in so far as mortality and morbidity rates are concerned. In no case, however, has a complete investigation been made which compares these cases of manual removal with those of a general clinic population. Accordingly, it seemed advisable to make such a study on the experience of the Obstetrical Service at the Johns Hopkins Hospital. The results of this analysis are given in the present paper.

From the opening of the Department of Obstetrics at the Johns Hopkins Hospital in 1896 to June 1, 1933, there were 186 cases in which manual removal of the placenta was performed following delivery of a viable child. This series, then, does not contain cases of manual removal prior to the seventh month of pregnancy, neither does it include instances in which the procedure has been included in a vaginal Caesarean section. Of the 186 patients, 171 were delivered in the hospital, while 7 were admitted for the operation following

delivery on the Outside Service and the 8 remaining were admitted after delivery of the child by a private physician or midwife in the patient's home. In only one instance was the operation done in successive deliveries of the same patient.

INCIDENCE

Table 1 indicates that the incidence of manual removal in patients delivered in the hospital of a viable child was 0.83 per cent or 1 in 120 cases for the entire series. It will be noted that in recent years the operation has been employed less frequently than in the early days of the Clinic. This difference is explained chiefly by the large number of cases of *accouchement forcé* with subsequent manual removal employed in the radical treatment of eclampsia and allied conditions in the earlier cases—a technique which has been gradually abandoned.

TABLE 1

Incidence of manual removal of placenta in terms of total patients delivered at or near term

	TOTAL CASES	EXCLUDING ADMISSIONS AFTER DELIVERY OF CHILD
1896–1909	1.63% or 1 in 61 delivered	1.46% or 1 in 68 delivered
1910–1919	0.97% or 1 in 103 delivered	0.94% or 1 in 106 delivered
1920–1932	0.76% or 1 in 132 delivered	0.68% or 1 in 147 delivered
1896–1933	0.91% or 1 in 110 delivered	0.83% or 1 in 120 delivered

Since 1920 the incidence, excluding the patients admitted after delivery of the child, has been 0.68 per cent; and further, excluding those cases in which a previous obstetric operation has been the indication for the subsequent manual removal, it is 0.48 per cent or 1 in 208 deliveries.

Before proceeding farther with the analysis it should be noted that in only 67 (36.22 per cent) of the 186 cases had the delivery been unaccompanied by any operative procedure, while in almost two-thirds of them forceps, version, breech extraction or some other type of manipulation had been employed. In 7 instances the pregnancy had been multiple.

EFFECT OF RACE, AGE AND PARITY

Table 2 indicates the incidence in the total number of cases of manual removal of the placenta, together with those following spon-

taneous delivery, as affected by race, age, and parity of the patient and as contrasted with the general clinic population. It will be seen that, although more black than white women are delivered in the Clinic, almost two-thirds of the cases of manual removal were in the white race. The reason for this very considerable disparity is not at all clear and affords still another example of the marked variation in obstetric reaction between the two races, a fact which we have frequently pointed out. Table 2 also indicates that the operation predominantly follows delivery of multiparous women as well as those in the later age periods of their childbearing careers. This difference is not to be explained in terms of frequency of postpartum hemorrhage since it has been found that the latter is noted more often among primiparae.

TABLE 2

Effect of race, age, and parity on the incidence of manual removal of the placenta

	GENERAL CLINIC POPULATION	MANUAL REMOVAL	
		Total cases	After spontaneous delivery only
Race:			
White	47.75%	63.44%	64.17%
Black	52.25%	36.56%	35.83%
Age:			
Mean age of patients	23.86 yrs.	28.91 yrs.	27.58 yrs.
Parity:			
Primiparae	54.69%	37.16%	37.88%
Multiparae	45.31%	62.84%	62.12%

DURATION OF LABOR

A study of the duration of labor excluding the third stage indicates that for the total number of cases of manual removal the mean duration is definitely prolonged in both primiparae and multiparae. In the primiparous group the prolonged labor is frequently terminated by some operative procedure rendering subsequent manual removal advisable, and in the cases delivered spontaneously the average labor is shorter by four hours than for the general clinic population. However, in the multiparous portion of the series the average spontaneous labor is definitely longer than in the general clinic experience, and it seems probable that in this group are to be found a number of instances of uterine inertia followed after delivery by uterine atony resulting

in imperfect separation of the placenta, hemorrhage, and manual removal. The facts noted above are still further illustrated by a consideration of the incidence of prolonged labor, *i.e.*, 30 hours or more, as is shown in table 3. It will be noted that prolonged labor occurred in 14.29 per cent of the cases of manual removal following spontaneous delivery in multiparae, an incidence four times as great as that for the general clinic population.

TABLE 3

Duration of labor

	GENERAL CLINIC POPULATION	MANUAL REMOVAL	
		Total cases	After spontaneous delivery only
Mean duration—primiparae	17.59 hrs.	23.21 hrs.	13.57 hrs.
Mean duration—multiparae	11.75 hrs.	16.20 hrs.	15.34 hrs.
Incidence of prolonged labor—primiparae	11.97%	29.82%	4.76%
Incidence of prolonged labor—multiparae	4.67%	16.84%	14.29%

DURATION OF THE THIRD STAGE AND POSTPARTUM HEMORRHAGE

The duration of the third stage of labor varied from a few minutes to three days after delivery, although all patients with a duration of over two hours were admitted to the service after delivery by outside agencies. In 30 per cent of the total number two or more hours elapsed between the birth of the child and extraction of the placenta. The mean duration of the third stage in the series was 40.31 minutes as contrasted with 9.93 minutes for the general clinic population.

Many of the case records gave no definite information as to the amount of bleeding after delivery, but obviously the incidence of postpartum hemorrhage was high and amounted to 60.29 per cent, whereas for the total clinic population it was 6.14 per cent. The average blood loss for the series was 832 cc., a figure about 500 cc. in excess of the general clinic experience.

It may be stated that tamponade of the uterus subsequent to manual removal of the placenta was practised in 20 per cent of the cases.

DURATION OF PREGNANCY

Among the cases in which the duration of pregnancy could be determined with a reasonable degree of accuracy there was a high incidence of prematurity as shown in table 4. It has been suggested that syphilis often necessitates manual removal of the placenta, but such was not the case in this series since only five of the placentae showed microscopic evidence of the disease. In almost a quarter of the cases analyzed delivery took place at least a month before term, which is out of all proportion to the general clinic experience.

TABLE 4

Duration of pregnancy

Premature—4 weeks or more	22.63%
Premature—2–4 weeks	10.22%
Term—2 weeks premature to 2 weeks postmature	55.47%
Postmature—2 weeks or more	11.68%

INDICATION

The indications for manual removal of the placenta may be divided into three groups: hemorrhage, retention, and operative delivery with the desire to complete the labor at once. An example of the last group was found in a number of cases in which, following version and extraction, it was felt necessary to explore the body of the uterus

TABLE 5

Indication for manual removal

	TOTAL CASES	AFTER SPONTANEOUS DELIVERY ONLY
Hemorrhage	94 or 54.34%	46 or 70.77%
Retention	25 or 14.45%	19 or 29.23%
Operation plus	54 or 31.21%	0 or 0.00%

in order to rule out rupture. This exploration, followed by manual removal, was done immediately after delivery of the child.

From table 5, 13 cases have been omitted because the history did not state definitely the indication for extraction of the placenta. It will be seen that for the total series hemorrhage was the indication in just over half the cases, and retention in about a seventh, the remainder

falling in the operation plus group. When delivery had been spontaneous, hemorrhage and retention were the causative agents in seven-tenths and three-tenths of the cases, respectively. It might be stated that the gross and corrected maternal mortality rates for the three groups were as follows: hemorrhage, 4.26 and 2.13 per cent; retention, 8.00 and 4.00 per cent; and operation plus, 25.93 and 7.41 per cent, respectively.

PUERPERIUM

The gross puerperal morbidity rate, omitting 9 patients who died within 48 hours of delivery, was 55.37 per cent.

Table 6 indicates the extremely high rate of puerperal infection when manual removal of the placenta is done. Undoubtedly if other obstetrical procedures precede the extraction of the placenta, they

TABLE 6

Puerperium

	MANUAL REMOVAL ALONE	MANUAL REMOVAL PLUS OPERATION
No puerperium (died within 48 hrs.)	1	8
Afebrile	20	33
Febrile, 1 day fever	11	15
Febrile, other causes	3	2
Febrile, puerperal infection	32 (48.48%)	61 (54.95%)

contribute largely toward the ensuing infection. However, it is noteworthy that when manual removal constituted the only manipulation practically half of the patients developed a puerperal infecton, which graphically indicates the dangers of the operation. These fatal cases of puerperal sepsis will be discussed under maternal deaths.

MATERNAL DEATHS

In this series of 186 cases of manual removal of the placenta, there were 20 maternal deaths, giving a gross mortality rate of 10.75 per cent. The causes of death, together with their classification, are shown in table 7.

The above classificaton has been made in an effort to derive a mortality rate due to the operation of manual removal itself. A brief résumé of the deaths in each group follows.

A. Group 1. Non-infective.

Cases 1 and 2. Eclampsia. Antepartum convulsions. Delivery by *accouchement forcé*, version, and extraction of placenta. Death due to shock and eclampsia.

Case 3. Placenta praevia. Delivery (early days of Clinic) by manual dilatation of cervix and version followed by removal of placenta. Death shortly after delivery due to hemorrhage.

Case 4. Placenta praevia. Insertion of bag, version and extraction, manual removal of placenta and tamponade of uterus. Death on first day due to hemorrhage.

TABLE 7

Maternal deaths following manual removal of the placenta

CAUSE OF DEATH		
Group 1. Non-infective		9 or 4.84%
Eclampsia	2	
Placenta praevia	2	
Nephritis	1	
Surgical shock	1	
Chloroform poisoning	1	
Ruptured uterus	1	
Hemorrhage (admitted moribund)	1	
Group 2. Infection—with other cause		5 or 2.69%
Lobar pneumonia	2	
Intrapartum septicemia	1	
Ruptured uterus—peritonitis	1	
Embolus—puerperal infection	1	
Group 3. Infection—no other cause		6 or 3.23%
Septicemia	2	
Peritonitis	2	
Thrombophlebitis	2	
Total		20 or 10.76%

Case 5. Admitted in early labor. Uremia. Manual dilatation of cervix, version and extraction, removal of placenta. Death on first day. Autopsy—nephritis.

Case 6. Induction of labor by balloon. Manual dilatation of cervix, version and extraction, removal of placenta. Death on operating table due to surgical shock.

Case 7. Delivery of dead child by craniotomy. Moderate bleeding, manual removal and tamponade of uterus. Death on second day from chloroform poisoning.

Case 8. Severe pyelitis. Delivery by difficult version. Manual removal followed by exploration of uterus revealing complete rupture. Hysterectomy and death within a few hours from hemorrhage and shock.

Case 9. Spontaneous delivery attended by outside physician. Immediate hemorrhage and transfer to hospital. Moribund on admission and death a few minutes later.

It seems clear enough that the operation of manual removal played no rôle in the causation of any of the deaths in this group. As has been noted, most of them occurred in the early days of the Clinic at a time when accouchment forcé was in use.

B. Group 2. Infection with other cause.

Cases 10 and 11. Lobar pneumonia. Premature spontaneous delivery, not induced. Retention of placenta with manual removal. Death a few hours later from fulminating septicemia.

Case 12. Intrapartum septicemia. Admitted with marked intrapartum infection. Breech extraction and manual removal. Death a few hours later from fulminating septicemia.

Case 13. Ruptured uterus. Peritonitis. Induction of labor by balloon. Version and extraction, manual removal of placenta. Exploration of uterus revealed complete rupture. Hysterectomy. Death on 9th day from peritonitis.

Case 14. Mild puerperal infection. Pulmonary embolus. Symphysectomy, forceps, and manual removal. Febrile puerperium, sudden death on 9th day from pulmonary embolus.

In this group it is felt that although the operation of manual removal may have had some effect in producing infection, yet it was a minor one in so far as the actual cause of death was concerned.

C. Group 3. Infection with no other cause.

Case 15. Septicemia. Toxemia. Induction of labor by balloon. Version and craniotomy. Manual removal of placenta. Temperature rose on second day, uterine and blood cultures showed a hemolytic streptococcus. Death on 14th day, septicemia.

Case 16. Peritonitis. Death of child *in utero.* Induction of labor by bougie. Craniotomy. Retention of placenta and manual removal. Temperature rose during labor. Death on 8th day—peritonitis.

Case 17. Thrombophlebitis. Placenta praevia. Balloon and version and extraction. Temperature rose on 4th day, uterine culture showed an anaërobic streptococcus. Death on 49th day due to thrombophlebitis.

Case 18. Septicemia. Spontaneous delivery. Profuse bleeding and manual removal of placenta. Temperature rose on 3rd day. Death on 12th day—septicemia.

Case 19. Peritonitis. Spontaneous delivery. Retention of placenta and manual removal. Temperature rose on third day, uterine culture showed B. coli and an unidentified streptococcus. Death on 6th day due to peritonitis.

Case 20. Thrombophlebitis. Spontaneous delivery. Profuse hemorrhage and

manual removal. Temperature rose on 4th day, uterine culture showed aërobic and anaërobic streptococci. Death on 70th day due to thrombophlebitis.

The deaths in this last group of cases were due solely to some form of puerperal infection. In three of them major obstetric operations were done in addition to the procedure of manual removal and probably played a part in the production of the subsequent infection. However, in the remaining three cases, extraction of the placenta was the only operation and it is reasonable to assume that it was directly responsible for the fatal infection.

In the 67 cases in which delivery had been spontaneous, the gross mortality rate was 10.45 per cent, a figure almost identical with that of the entire group. As has been stated above, three deaths due solely to infection occurred in this group, giving a corrected death rate of 4.48 per cent.

DISCUSSION

An analysis of 186 cases of manual removal of the placenta at the Johns Hopkins Hospital gives confirmatory evidence of the dangers of the procedure from the point of view of puerperal sepsis. The operation should never be undertaken lightly and a rigid standard should be assumed in so far as the indications for and technique of the extraction are concerned. Obviously, the absolute necessity for performing this operation will always exist and the operation often will have to be done on patients who are not ideal surgical risks. Because of the inherent dangers in the operation we feel that its indications must be clear-cut and urgent. It is the opinion of this Clinic that there are only three groups of circumstances which justify manual removal of the placenta.

1. *Hemorrhage.* In the presence of free bleeding, associated with partial separation of the placenta, extraction will often be necessary. Frequently the partial placental separation will be the result of improper treatment of the third stage of labor, since massage of the fundus and premature attempts at expression before separation of the placenta has occurred will often give rise to bleeding which otherwise would have been preventable. It seems reasonable to assume that a large number of cases of manual removal could be avoided if a "hands off" policy be employed during the third stage in the absence of bleeding and if the separation of the placenta be left to its normal mech-

anism. However, it should be remembered that if the patient has had any considerable amount of antepartum hemorrhage she will not tolerate an excessive amount of bleeding after delivery so that in cases of placenta praevia or premature separation, if bleeding occurs and the placenta cannot be promptly expressed, manual removal should be undertaken with much less hesitation. Obviously, no exact criterion can be given as to the amount of hemorrhage after delivery which will afford an indication for extraction, since it must depend entirely upon the circumstances of the individual case.

2. *Retention.* The average duration of the third stage of labor in this Clinic, with its policy of avoiding if possible any manipulation until the placenta has separated, is 10 minutes. In only 19 instances was retention the indication for manual removal and 3 of these were in patients delivered in their homes by private physicians, giving an incidence in terms of cases delivered in the hospital of 1 in 1,250. With the patient in good condition and in the absence of bleeding it is believed that manual removal should not be attempted until two hours have elapsed following delivery and conservative methods of promoting separation have all failed.

3. *Previous obstetric operations.* No definite rule can be laid down for this group except to state that it should be limited in so far as is possible. Obviously, major obstetric procedures culminating in delivery from below often leave us with a patient whose interests will best be served by immediate termination of the third stage of labor in order that treatment of shock or previous hemorrhage may be started at once under more favorable conditions. Also, following a difficult podalic version and extraction, exploration of the uterine cavity is frequently advisable and manual removal of the placenta is indicated as a concomitant procedure. Generally speaking, however, it seems preferable to disregard previous operative procedures and to demand in these cases the same indications for extraction of the placenta.

Finally, we wish to deplore the frequent tendency on the part of many obstetricians to employ tamponade of the uterus as a routine procedure following manual removal. Obviously this practice increases the occurrence of puerperal infection. It is felt that the two procedures should be entirely independent of each other in so far as their indications are concerned.

CONCLUSIONS

1. The incidence of manual removal of the placenta in women delivered at or near term at the Johns Hopkins Hospital from 1896 to 1933 was 1 in 120 cases.

2. This operation was necessary much oftener in white than in black patients delivered in the Clinic. It also was performed more commonly in multiparae and in women during the older age periods of their childbearing careers.

3. The incidence of manual removal was higher following prolonged labor and was particularly common in multiparae delivered spontaneously after a long labor due to uterine inertia.

4. Manual removal was necessary much more frequently following premature than term delivery.

5. Hemorrhage was the most common indication for the procedure and retention of the placenta the least common. In about a third of the cases a previous obstetric operation with a desire to terminate the third stage promptly was given as the indication for manual removal.

6. The gross morbidity rate excluding cases dying within 48 hours of delivery was 54.95 per cent. After spontaneous delivery, and where manual removal was the only manipulation, in 48.48 per cent of the patients there followed a febrile puerperium.

7. The gross maternal death rate was 10.76 per cent and that due to sepsis 3.23 per cent. In cases where delivery was spontaneous the above two rates were 10.45 and 4.48 per cent, respectively.

8. The operation of manual removal of the placenta has its definite indications but its employment should be limited. Intelligent care of the patient during the third stage of labor will lower its incidence by preventing partial separation.

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CARDIAC ARRHYTHMIA IN EXPERIMENTAL SUPRARENAL INSUFFICIENCY IN DOGS[1]

WILLIAM M. NICHOLSON, M.D.,[2] AND LOUIS J. SOFFER, M.D.

From the Chemical Division of the Medical Clinic, the Johns Hopkins University and Hospital

Submitted for publication February 12, 1935

During the course of the studies on suprarenalectomized dogs carried out in this laboratory in the past few years, it has been observed that a considerable proportion of animals which are allowed to develop symptoms of insufficiency, by withdrawal of injections of cortical extract or by the administration of insufficient amounts of salt, exhibit a very slow heart rate. Stewart and Rogoff (1) in 1926 made note of the bradycardia and gave protocols of several experiments on animals. They offered no explanation of the phenomenon.

We have been able to find twenty-two experiments in the series of suprarenalectomized dogs in which bradycardia occurred when the animal was allowed to lapse into insufficiency. No doubt this has happened more frequently, but no note was made of the fact at the time.

Table I shows the findings in 14 dogs in which bradycardia occurred but in which an electrocardiogram was not obtained. In all these cases the heart rate was 65 or less and in 5 cases it was below 40; the blood non-protein nitrogen was 85 mgm. per cent or more, and the plasma sodium and chloride concentrations were below normal, except in two instances. Potassium determinations of the plasma were done on two of these animals and in both they were found to be markedly elevated.[3]

[1] The expenses of this investigation were aided in part by the William Bingham Fund.

[2] John D. Archbold Fellow in Medicine.

[3] The routine employed in obtaining blood for analysis, and the methods of analysis, are given in a previous communication from this laboratory (5). The potassium determinations were made according to the method described by Kerr. (Kerr, S. E., *Jour. Biol. Chem.*, 1926, **67**, 689.)

TABLE I

Dogs exhibiting slow heart rate, without electrocardiographic studies

DOG	DATE	HEART RATE	N.P.N.	SODIUM	CHLORIDE	POTASSIUM	REMARKS ON THE PRODUCTION OF INSUFFICIENCY
		per minute	*mgm. per cent*	*m.eq./l*	*m.eq./l*	*m.eq./l*	
F 7	12/16/31	60	96	127	104	—	Received no extract or sodium chloride for 7 days
W 13	5/13/31	48	140	—	—	—	No extract or sodium chloride for 6 days
F 29	4/28/32	44	148	—	—	—	No extract for 5 days
F 38	9/30/32	28	136	—	408*		Insufficient extract; no sodium chloride
56	12/14/32	48	85	—	452*	—	Insufficient extract; no sodium chloride
60	3/27/32	"Slow"	96	—	—	—	Insufficient extract; no sodium chloride
60	5/13/32	"Slow"	74	147	116	—	Insufficient extract; no sodium chloride
67	12/13/32	42	196	—	540*	—	No extract or sodium chloride for 7 days
103	12/17/33	26	146	136	103.3	—	Insufficient extract and sodium chloride
108	10/14/33	66	100	123	97.8	—	No extract or sodium chloride for 5 days
111	10/ 1/33	50	120	—	—	—	Insufficient extract and sodium chloride
129	3/30/34	38	84	141.7	111.4	17.8	Sustained with sodium chloride alone for 31 days
131	5/ 9/34	64	84	146	125	19.3	Sustained with sodium chloride for 21 days

* Whole blood, reported in mgm. per cent.

TABLE II

Dogs upon which electrocardiographic studies have been made

DOG	DATE	HEART RATE	N.P.N.	SODIUM	CHLORIDE	POTASSIUM	REMARKS ON THE PRODUCTION OF INSUFFICIENCY
			mgm. per cent	*m.eq./l*	*m.eq./l*	*m.eq./l*	
W 4	11/20/30	40	250	—	344*	—	Insufficient extract; no sodium chloride
118	12/27/33	60	108	134.4	101	—	Insufficient extract and sodium chloride
113	12/28/33	60	140	138	102	—	Insufficient extract and sodium chloride
	1/30/34	62	62	147	121	13.3	9 days without extract and with insufficient sodium chloride.
113	1/30/34	90					Reverted to normal rhythm and rate following injection
	1/31/34	40					of 200 cc. of normal saline. 24 hours later was again
	2/ 1/34	40	112	150	127	19.3	fibrillating, but reverted after the injection of the same
							amount of normal saline. 48 hours after the initial
							electrocardiogram he was again fibrillating
132	3/17/34	40	66	150.1	129.2	18.3	Sustained with sodium chloride alone for 27 days
132	5/21/34	40	112	134.2	106.3	23.6	Sustained with sodium chloride alone for 17 days
145	10/26/34	32	80	131.6	112.9	16.5	Sustained for 22 days with sodium chloride alone
141	11/21/34	60	176	130.2	88.9	14.5	Insufficient extract

* Whole blood, reported in mgm. per cent.

In addition we have obtained electrocardiographic tracings on 8 dogs which exhibited slowing of the heart rate during suprarenal insufficiency, and we have also studied simultaneously the plasma electrolyte pattern of six of these animals. The electrocardiographic tracings (fig. 1) of all are exactly alike, except for the variation in the

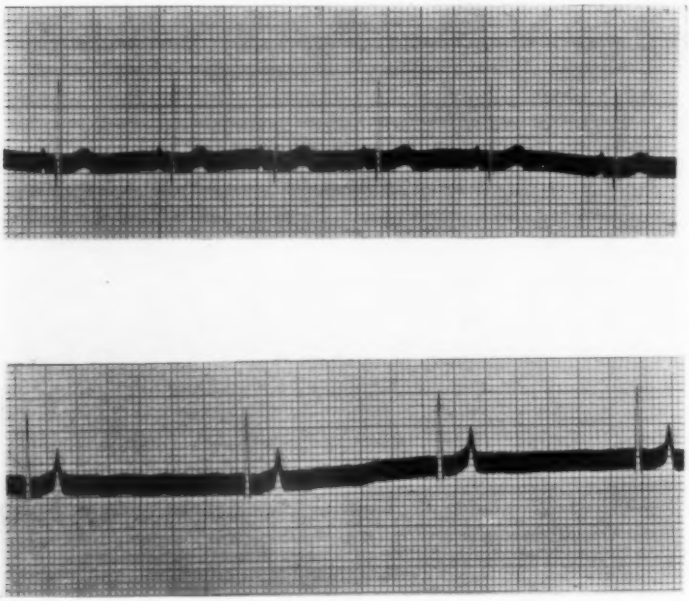

FIG. 1. ELECTROCARDIOGRAPHIC TRACINGS ON DOG 145

The lower record was taken while the animal was in suprarenal insufficiency. The one above was made one week later when the animal had been given sodium chloride and cortical extract.

rate. The rate in no instance was more than 60, and in one case was as low as 32. The tracings show a totally irregular rhythm with an absence of the P waves, and are interpreted as showing auricular fibrillation.

The plasma potassium was in no instance below 13.3 m.eq./l and in one animal was found to be 23.6 m.eq./l (table II). The plasma

sodium and chloride concentrations were low, normal, or slightly elevated. Those animals in which the plasma sodium and chloride concentrations were elevated were given sodium chloride by mouth and parenterally when the first symptoms of insufficiency appeared. When haemoconcentration has become so marked that insufficient water is available for the excretion of electrolytes, the sodium and chloride of the plasma may be increased, and this fact may explain the apparent paradox occasionally observed of typical suprarenal insufficiency with high plasma sodium and chloride values. This effect is analogous to that noted by Hartman, (16) who observed that when urinary excretion becomes too scanty, as in the anhydremia of diarrhoea in infants, the total electrolyte concentration in the plasma occasionally reaches enormous values.

When sodium chloride was given intravenously in large quantities, the cardiac mechanism reverted immediately to a normal rhythm and rate. One animal (Dog 113) which showed the characteristic changes, was given 200 cc. of normal saline, following which there was reversion to normal rhythm; 24 hours later the heart rate was 40 and an electrocardiogram taken at this time showed that fibrillation was again present. Following the intravenous injection of 200 cc. of physiological salt solution the animal again reverted to normal rhythm. Dog 141 also exhibited auricular fibrillation and reverted to normal rhythm immediately after the injection of 150 cc. of 1 per cent sodium chloride.

DISCUSSION

In experimental suprarenal insufficiency there are certain characteristic changes in the composition of the blood. Britton reviewed the subject in 1930 (2), and up to that time the changes which had been particularly noted were an increase in blood urea and non-protein nitrogen concentrations and haemoconcentration. It has since been repeatedly shown that when suprarenalectomized animals are allowed to develop symptoms of insufficiency, there is a disturbance in the plasma electrolyte pattern, consisting of a reduction in total base, sodium bicarbonate and chloride values and an increase of potassium (3, 4, 5, 6, 7, 8).

From data shortly to be published elsewhere it can be shown that

the level of plasma potassium follows consistently the level of the blood urea concentration during suprarenal insufficiency in dogs. So far as we have been able to find, this correlation between the potassium and the urea concentration is constant in experimental suprarenal insufficiency. We can therefore assume that in the above mentioned cases in which bradycardia and an elevated blood urea were both present, there was also an elevation of the plasma potassium.

Hastings and Compere (8) in 1931 reported that the plasma potassium concentration was increased in experimental suprarenal insufficiency in dogs. They suggested that some of the symptoms following suprarenal extirpation might be explained by potassium poisoning, inasmuch as the evidences of suprarenal insufficiency occurred at about the level of plasma potassium concentration at which toxic manifestations appear. It has been known for many years that potassium has a marked effect on the cardiac mechanism. This effect may be produced either by intravenous injection, or in the perfused, isolated heart. Blake (9) in 1839, Traube (10) in 1864, Aubert and Dehn (11) in 1874, Mathison (12) in 1911, Wiggers (13) in 1930, and others, have demonstrated the changes that occur following the injection of potassium chloride. While there is difference of opinion as to the necessary dosage, all observers agree that either auricular or ventricular fibrillation may occur after the injection of this substance. In two experiments that were made in our laboratory the following results were obtained: the first dog was given 100 cc. of 1 per cent potassium chloride in the jugular vein. The injection extended over a period of 45 minutes. This represented 66 mgm. per kilogram of body weight of the animal. The only disturbance that occurred in the cardiac mechanism was sinus arrhythmia and slowing of the rate. Another animal was given 200 cc. of a 1 per cent solution of potassium chloride within 16 minutes. This amount was equivalent to 133 mgm. per kilogram of body weight, or if we assume the total blood volume to be 1000 cc., an actual increase of 27 m.eq./l in the plasma potassium. The animal promptly developed auricular fibrillation, followed immediately by ventricular tachycardia and death.

Blum and others (14) have shown that when potassium chloride is injected into the vein of a normal man, the tissues immediately take up the potassium, so that a large amount must be given in order to

produce a rise in the concentration of blood potassium. Less potassium chloride however is necessary to produce this rise when the individual is in a moribund condition. We think, therefore, that in the first animal, when the potassium chloride was given slowly, the tissues of the body immediately took up the potassium and the effect on the heart was not fully developed. In the second animal, however, the concentration was great enough to produce auricular fibrillation and finally death. In suprarenalectomized dogs that develop insufficiency the tissue water has a high potassium concentration. The kidneys are incapable of excreting the excess potassium and as a result there is a marked accumulation of potassium in the blood plasma. This phenomenon is not precisely comparable to the conditions existing in those animals in which potassium chloride is injected intravenously. In the animals exhibiting suprarenal insufficiency there is a slow accumulation of potassium in the tissues and in the blood, while in the normal animal, after injection, the concentration is initially very high in the plasma and then falls rapidly as the potassium is transferred into the tissues. The constantly elevated potassium in both the plasma and tissues, we believe, is responsible for the characteristic disturbance in the conduction mechanism.

There is no significant disturbance in the calcium metabolism in experimental suprarenal insufficiency. The slight rise that does occur is explained on the bases of haemoconcentration, and parallels the rise in plasma protein to which calcium is bound. It was, however, desired to determine the effect of calcium on the cardiac mechanism in animals showing auricular fibrillation during suprarenal insufficiency. For this purpose one dog in which auricular fibrillation occurred during suprarenal insufficiency was given 10 cc. of 5 per cent calcium chloride intravenously. The animal immediately went into collapse, the heart rate fell to about 4 beats per minute and electrocardiograms taken during this period showed that auricular fibrillation was still present. Subsequently he recovered and following the injection of sodium chloride, the heart reverted to normal rhythm and rate.

Auricular fibrillation is not characteristic of the terminal stages of Addison's disease. In the studies reported by Loeb, however, (15) and according to our own observations, there is no rise in the plasma

potassium comparable with the rise we have found in experimental suprarenal insufficiency in dogs. Sampayo and others (22) report electrocardiographic studies in Addison's disease, and find that there is a low voltage with prolongation of the S-T interval, but in no case was auricular fibrillation present.

That the cortical hormone has no specific effect in bringing about or relieving the cardiac irregularity observed in suprarenal insufficiency in dogs is shown by the fact that when the animals are given sodium chloride alone, they revert to a normal rhythm. It is our belief that the auricular fibrillation is produced by the disturbance in the relationship of the ions of the plasma.

SUMMARY

1. A characteristic disturbance in the cardiac action of the dog is reported in experimental suprarenal insufficiency. This disturbance is manifested by a slow auricular fibrillation.

2. Reasons are advanced for the view that the disturbance is due to the distortion of the plasma electrolyte pattern and especially to the high concentration of potassium.

3. A comparison with Addison's disease is made.

We are indebted to Miss Julia Ann Vickers of the Cardiographic Laboratory for taking the electrocardiograms, and to various members of the staff of the Chemical Laboratory for assistance in technical procedures. To Dr. Edward P. Carter we owe many thanks for aid in the interpretation of these studies and of the electrocardiographic findings.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

The Physiological Effects of Radiant Energy. By HENRY LAURENS. [American Chemical Society. Monograph Series.] 1933. 610 pp. $6.00. (*Chemical Catalog Co., Inc., New York.*)

A very comprehensive review of the physiological effects of radiant energy on the skin, eye, blood and metabolism. The author's plan of a source book of information has been thoroughly executed and his bibliography is very complete. Due to variations in experimental method, the results of different observers are often contradictory and no attempt has been made to draw conclusions or to make a critical analysis of the findings.

J. H. C.

Practical Neurological Diagnosis. By R. GLEN SPURLING. 1935. 233 pp. $4.00. (*Chas. C. Thomas, Springfield, Ill. & Balto., Md.*)

A concise, careful exposition of the methods of neurological diagnosis now available. There are numerous, helpful diagrams, x-rays and photographs, along with lucid descriptions of technique. Ventriculography and encephalography are discussed in detail. Altogether, a very valuable book.

W. T.

Modern Drug Encyclopedia and Therapeutic Guide. By JACOB GUTMAN. 1934. 1393 pp. $7.50. (*Paul B. Hoeber, Inc., New York.*)

A very useful reference book including the composition of remedies with a trade name that gives no inkling of the formula. Perusal of the book will convince the reader that, in spite of great efforts made to bring therapeutics to a high and rational level, many worthless remedies are still being foisted on the public. The author has rendered valuable service in assembling thousands of medicinal preparations for ready reference.

E. M. K. G.

The Pathology of the Eye. By EUGENE WOLFF. 1935. 282 pp. $7.50. (*P. Blakiston's Son & Co., Inc., Philadelphia.*)

This volume offers a fairly complete outline review of the subject, with a profusion of illustrations. The presentation is impersonal. The material is arranged according to the anatomical sites of the lesions, with additional chapters on refraction, strabismus and general pathology. The omission of corneal dystrophy is regrettable, and in the section on the vitreous no clear distinction is made

between asteroid hyalitis and synchisis scintillans. Apart from some misspelling of proper names, the volume is relatively free from minor errors. An adequate bibliography is appended.

E. P. B.

Nutrition and Disease. By EDWARD MELLANBY. 1934. Illust. 171 pp. 8/6. (*Messrs. Oliver & Boyd, Ltd., London.*)

This book represents three Croonian Lectures delivered before the Royal College of Physicians in 1933. The author discusses, in the light of modern research, rickets, dental structure and disease, the thyroid gland, nutrition and infection, nutritional influences on the nervous system (experimental work on animals), and extension of experimental results on nerve degeneration to certain other clinical conditions. Under the last caption are discussed beri-beri, convulsive ergotism, pellagra, lathyrism, subacute combined degeneration of the cord, and disseminated sclerosis.

These lectures constitute a scholarly presentation of the interaction of clinical and experimental work in fields in which the writer has made notable contributions as an investigator, while holding a combined laboratory and clinical post. They conform to the high standard which has characterized the previous contributions of this distinguished investigator.

E. C. McC.

The Lyophilic Colloids. Their Theory and Practice. By M. H. FISCHER and M. G. HOOKER. 1933. 246 pp. $4.50. (*Chas. C. Thomas, Springfield, Ill. & Balto., Md.*)

This volume is neither a "Text-Book" nor a "Monograph," but more or less of a compilation of Fischer's previously expressed views on the subject, with no review of the work of others in the field. Those who have followed Fischer's work will find little new in it, but the novice may discover extremely interesting, though unorthodox ideas concerning the rôle of the colloids in biologic systems. As in the case of Fischer's "Edema and Nephritis," such views may find but few adherents among biologists and physiologists, while younger students of biophysics and physical chemistry may discover ideas and suggestions lurking in every nook and corner. Such ideas must be translated into modern concepts, instead of the all important pH, to explain the colloidal behavior of the living cell.

D. H. S.

Cataract. Its Etiology and Treatment. By CLYDE A. CLAPP. 1934. 254 pp. $4.00. (*Lea & Febiger, Philadelphia.*)

A volume of 254 pages divided into 25 chapters. Chapters I–VII cover the embryology, comparative anatomy, nourishment, physiology, chemistry and congenital anomalies of the lens, the first two chapters being by Dr. Ida C. Mann. Chapters VIII–XXV cover the etiology, complications and treatment of cataract.

The individual chapters are of a disappointing brevity, which, however, is compensated for by the well chosen and comprehensive bibliographies appended. Thus the book gives the highlights, with ready access to the voluminous literature. Post-operative complications are adequately discussed, but operative complications receive little mention. Apart from the errors common to most first editions, the texture is accurate and bears evidence of painstaking revision. The illustrations are satisfactory. While many cuts have been borrowed from other text-books and articles, a number are original drawings by Mrs. Burgess.

The volume fills a definite want in ophthalmologic literature, and should prove useful to the ophthalmic surgeon.

A. C. W.

BOOKS RECEIVED

Der Aerzte-Kniffe. 1935. 100 pp. Rmk. 2.60. Georg Thieme, Leipzig.

Textbook of Anatomy & Physiology. By DIANA C. KIMBER, CAROLYN E. GRAY & C. E. STACKPOLE. 1934. 9th ed. Illust. 640 pp. $3.00. Macmillan Co., New York.

Pflanzliche Therapie. By ERNST MEYER. 1935. 207 pp. Rmk. 4.80. Georg Thieme, Leipzig.

Mary Imogene Bassett Hospital. Clinical Miscellany. 1934. Vol. 1. 204 pp. Illust. $3.00. Charles C. Thomas, Springfield, Ill. & Balto., Md.

The Rabbit Ovulation Test. By S. B. ANKLESARIA. [Reprinted from the Journal of the Univ. of Bombay, vol. 1, pt. v, March, 1933.] 1933. 169 pp.

Bailey's Textbook of Histology. By ADOLPH A. M. ELWYN & OLIVER S. STRONG. 1932. 8th edition. 746 pp. Wm. Wood & Co., New York.

Human Biology & Racial Welfare. By 28 authors. Edited by E. V. Cowdry. 1930. 612 pp. $6.00. Paul B. Hoeber, Inc., New York.

Principles of Preoperative & Postoperative Treatment. By REGINAL A. CUTTING. With a foreword by Rudolph Matas. 1932. 812 pp. $10.00. Paul B. Hoeber, Inc., New York.

Mother's Guide When Sickness Comes. By ROGER H. DENNET & EDW. T. WILKES. 1934. 400 pp. $2.50. Doubleday, Doran & Co., New York.

Syllabus of Psychiatry. A Guide to General Orientation. By LELAND E. HINSIE. 1933. 344 pp. $2.50. State Hospitals Press, Utica, N. Y.

The Expectant Mother's Handbook. By FREDERICK IRVING. 1932. 203 pp. $1.75. Houghton Mifflin Co., Boston, Mass.

The Recovery of Myself. By MARIAN KING. 1931. $2.00. Yale Univ. Press, New Haven, Conn.

Cancer, Its Origin, Its Development, & Its Self-Perpetuation. 1931. 427 pp. $7.50. Paul B. Hoeber, Inc., New York.

Oxidation-reduction Potentials. By L. MICHAELIS. Translated from the German Manuscript by Louis B. Flexner. 1930. 197 pp. $3.00. J. B. Lippincott Co., Philadelphia.

ERRATA

On pages 153 and 154 of article by McEachern in the March issue the legends of Figures 1 and 2 have been transposed.

For Fig. 1 read: Typical Curve of Oxygen Uptake by Tissues from a Normal and from an Hyperthyroid Animal.

For Fig. 2 read: Method of Studying Total Metabolism of Animals.

In Table XI, column 10, line 9, for +501 read +50.

5

STUDIES ON VIRUS PROBLEMS

I. Tissue and Cell Affinities of Viruses and Reactions of the Host[1]

J. C. G. LEDINGHAM

Director, Lister Institute, London

Let me in the first place express my high appreciation of the honour which the Advisory Board of your Medical Faculty do me and, may I add, the Institute I represent, by their cordial invitation to deliver the Herter Lectures.

While I gladly accepted the responsibility of addressing you, the manner and substance of my address gave me much concern when I reflected on the now vast field which the study of viruses and virus diseases occupies on the laboratory stage and on the fact that in this country, as in England, that field is being very assiduously tilled.

Acting on Professor MacCallum's suggestion I propose to give some account in these lectures of my own work and that of various colleagues in recent years in the field of virus research, but before I pass to the main subject of this first lecture I should like to give some consideration to the more general aspects of the problem and to offer some appraisement of the position we have reached as the result of this now world-wide attack on the virus stronghold. It is remarkable, I think, that Jenner and Pasteur laid such a sure foundation for the future intensive study of viruses in the 20th. century by their work on variola and rabies without their being troubled too acutely by the nature of the infective agents they were dealing with. Jenner, at any rate, had very clear views as to the dangers to which human beings might render themselves subject by living in too close relationship with animals. The famous "Rejected Inquiry" presented to the Royal Society in 1796 opened with the statement "The deviations of man from the state in which he was originally placed by nature seem to have proved a prolific source of diseases. From the love of Splendour,

[1]Lecture I (abridged) of the Herter Lectures, The Johns Hopkins University, Baltimore. Delivered December 5, 1934.

from the indulgence of Luxury and from his fondness for Amusement he has familiarised himself with a great number of animals which may not originally have been intended for his associates"—a prophetic remark, you will agree, when we reflect on the many virus diseases of animals now known to be communicable to man.

When Pasteur embarked on current problems of veterinary pathology which he was set to solve, he did so with one object in view, *viz.*, to prevent their occurrence by exploiting the principle which his predecessor Jenner had proved good—the principle of the attenuated virus. Jenner and Pasteur were essentially practical people and it is true to say that the methods evolved by them for the prevention and control of variola and rabies stand today in a position little removed from their original form. They afford indeed one of the best illustrations of the fact that though the actual infective agent of a disease may escape observation in the general mass of the lesion associated with it, this circumstance is no real bar to the effective prosecution of research into its bionomics, pathogenic properties and immunising activity. The quarter of a century which followed Pasteur's death was dominated by achievements in the frankly microscopic world resulting from the pure culture methods of Koch and, may I add, of Lister whose contributions to the problem of pure culture are sometimes forgotten. Consequently, during this period the investigation of diseases whose infective agents could be seen and cultivated naturally held the field, while what we now call virus diseases, for lack of a better term, had to wait for other times and other methods.

Today the number of virus diseases known and studied is very large and additions to it come by no means infrequently.

Let us consider some of the leading criteria which, taken together, connote a virus as we understand the term or which suggest virus action.

Filterability, since the discovery of this property among the plant mosaics and in foot and mouth disease, was accepted as a working hypothesis for all viruses, but in the actual application of the test to various viruses in crude suspension contradictory results were so frequently obtained with different filters and different methods of testing activity of filtrates that in many cases filterability could not be definitely postulated as an invariable attribute. Possibly of all the

many viruses studied in these post-war years vaccinia has received the most attention and yet it is only within the last five years that this virus has been definitely acknowledged to be a filterable virus. The chief reason in my opinion for the contradictory results in the past with this virus was the lack of an accurate and reliable method for testing the presence of potent virus in a filtrate. The use of the dermis of the rabbit removed this difficulty, while further study has shown that only more porous Berkefeld filters such as the V. filter, but not the Seitz filters, let the virus through when presented to the filters in the form most likely to reduce adsorption to the substance of the filter. Now filterability is regarded as an essential attribute of all viruses and all it implies is simply that the agent has a dimension at or below the level of microscopic resolution, say 200$\mu\mu$ and under. Yet it must be remembered that filterability by itself does not necessarily connote a virus, as has been shown by studies on the genus Leptospira and by recent investigations on these peculiar filterable organisms for long regarded as viruses—pleuropneumonia and agalactia. To these I shall refer in my second lecture. Estimates of the actual dimensions of virus bodies based on their capacity to pass membrane filters of known porosity, have been made by Elford and his associates, while in the case of some viruses parallel investigations of size have been made by Bechhold with the aid of the high speed centrifuge. Probably the estimates arrived at are to be considered approximate only and not absolute, but even as approximate estimates they help us to envisage the wide range of size among the various viruses studied. Where the smaller viruses are concerned, those in the range of 10$\mu\mu$ to 100$\mu\mu$, it is probable we shall have to await the application of improved physical methods to which I shall later refer. So far as the larger viruses are concerned, which lie in the region of 100$\mu\mu$ to 200$\mu\mu$ or 250$\mu\mu$, the measurements by filtration and by the centrifuge give little more information than can be obtained by estimates of size based on direct measurement of the elementary bodies concerned in properly prepared stained preparations. Work on these elementary bodies, which has occupied myself and some of my colleagues in the past three years and extended in various directions will form the subject of my third lecture and I would just remark here that the methods which have been devised to permit the study of these bodies in pure suspension must

inevitably lead to some considerable repetition of much previous virus work carried out with cruder material. Nevertheless, the full recognition of filterability in some degree of all agents classed as viruses has introduced far greater precision into modern virus work and has been accompanied by the widest search for experimental animals likely and unlikely to be susceptible, often with surprising results, as witness the communicability of dog distemper to ferrets and of yellow fever and human and swine influenza to mice. We have now to think of susceptibility as dependent often on a particular route of introduction of the virus and it is no hindrance to the further study of the virus in these experimental animals, that their clinical reactions bear little resemblance to those of the naturally acquired human or animal disease.

In addition to filterability which simply determines a matter of dimension, another peculiar feature of virus infection and one, the first observation of which dates back more than half a century, is the occurrence in the lesion of what has become known as the cell inclusion. The nature of the intracytoplasmic inclusion, *e.g.*, in fowlpox and vaccinia, remained really quite obscure till quite recent times—until in fact Goodpasture in this country showed that a single Bollinger body was capable of initiating infection. Goodpasture's observation coupled with the fact that fowlpox virus was filterable, though admittedly not easily filterable, made it almost certain that the Bollinger body was likely to contain the actual virus in some particulate form. Being then (1930) in the midst of my early attempts to demonstrate by staining methods, and to convince myself of the reality and identity of the elementary bodies observed by Borrel in 1904 in fowlpox material and by Paschen in 1906 in that of vaccinia, I was able, like Goodpasture, to secure with the simplest technique preparations showing that the Bollinger body was composed of an aggregation of Borrel bodies and in fact was an intracellular colony of the virus agent. From that time date my interest and my investigations directed to the securing of pure suspensions of these elementary bodies for serological and infectivity tests. The Guarnieri body, as seen best in the epithelial cells of the rabbit cornea after scarification with smallpox or vaccinia virus, is a much smaller body and much less readily amenable to analysis by the film staining technique as applied to the

Bollinger body. There is, however, little doubt—and Goodpasture has provided some positive evidence in this direction from his work on the cultivation of vaccinia virus in the chick embryo—that the Guarnieri body is, like the Bollinger body, composed of the elementary bodies of Paschen. Complete proof is yet wanting however. In recent years similar intracytoplasmic inclusions have been demonstrated with ease in the epithelia of the skin of the mouse after skin inoculations with the virus of ectromelia. In 1930 I was able to demonstrate, by the usual staining methods applied to elementary bodies, the presence of great numbers of such bodies in material from skin lesions. Later it was shown, as might indeed have been expected from analogy with fowlpox, that the ectromelia inclusion of Marchal was really made up of the elementary bodies constituting the actual virus agents. In Psittacosis somewhat similar aggregations of the elementary bodies associated with this virus disease have been found to occur in endothelial cells in Giemsa-stained film preparations of the infected mouse spleen. Bedson, in fact, has shown that, in the early days after infection of the mouse with this virus, the elementary bodies included in these cells undergo an increase in size possibly of similar import to the well known increase in size of bacteria in the early hours of growth after seeding. These observations are of considerable importance as indicating the occurrence of dynamic changes in bodies which, as a rule, give the appearance of being uniformly static in size and shape.

Another type of inclusion which has of late received a good deal of attention is the intranuclear acidophilic body as it appears in a large series of virus infections such as herpes febrilis and zoster, chickenpox, yellow fever, virus III, laryngo-tracheitis of chickens and various forms of nervous disturbance associated with recently studied viruses such as Pseudorabies, B. virus and Equine encephalomyelitis. There would seem, in fact, to be a tendency to the formation of this type of inclusion in viruses which possess neurotropic tendencies and evidence is accumulating that such changes in the nuclei of nerve cells may be one of the earliest manifestations of direct virus attack on these cells. In the case of the intranuclear body there is not the same unanimity of opinion with regard to its nature as in the case of the intracytoplasmic body. How far these bodies represent new extraneous matter

or simply modified chromatic substance or a mixture of both has not been made clear by the staining methods of the cell morphologist or by the microincineration methods recently applied to the problem of their structure. That they do indicate the associated presence of a virus infection whether active or entirely latent is, however, usually admitted and they may in the latter case be the only evidence of the existence of such infection. Their development in tissue cultures in which the corresponding virus is being cultivated is a valuable guide to what is happening in such cultures and we know that their appearance may be inhibited if the cells are protected from the action of the virus by the presence of the corresponding antiviral serum. In a recent review of the subject of tissue changes in virus diseases I have taken the standpoint on the evidence available that it is unsafe to postulate from the occurrence of cell inclusions of whatever type and from the hitherto unsuccessful attempts to cultivate viruses *in vitro* in cell-free media (a question to which I shall refer in my second lecture) that viruses are essentially intracellular parasites and must, after introduction into the body, promptly obtain the shelter of cells whose ferment-complex is necessary to them to provide energy for their survival and multiplication. It is just possible that to some extent the intracellular habitat may be a mere accident in the life history of the virus in the host. In any case we require far more ample evidence than we now have as to the behaviour and movements of viruses, considered as elementary bodies, after their entrance into a susceptible host. Such evidence can come only from improvements in microscopical staining technique and I do not think that in the case of the larger viruses the problem is one incapable of solution. We have no knowledge of toxic action exerted by viruses which may assist them to attack susceptible cells whose metabolic products are necessary for the continued growth of the virus, and which would enable the virus to multiply for a brief period at least in the free tissue spaces. The possibility of this happening should not be lost sight of. As a matter of fact we have what would seem to be very strong analogies to the cell inclusion in the well known symbiosis of Leishmania parasites and lepra bacilli with mononuclear cells of the reticulo-endothelial system and with the massed occurrence of the minute organisms of Tularaemia in the parenchymatous cells of the liver. The actual

demonstration of virus agents forming an intranuclear inclusion has been forthcoming only in the case of Rocky Mountain Spotted Fever, as recorded by Pinkerton and Hass (1932). At the moment, however, it is not clear whether typhus fever and its congeners associated with Rickettsia bodies are to be included among the filterable viruses. There would seem to be quite a large number of instances in man and other animals in which inclusions of the intranuclear type occur quite accidentally and apart from clinical disease. One of these, the so-called salivary gland disease of Cole and Kuttner, has been shown to be associated with a virus, and I think it is the only one. Recently I note another case of this kind has to be added to the list, *viz.*, the occurrence of intranuclear inclusions in the salivary glands of Talpa (by Rector and Rector). This curious field of enquiry is going to tax virus experts for some time to come.

I pass now to consider certain special cases of cell and tissue tropisms among the viruses because it is a subject of very great importance at the moment for the understanding of the routes of infection traversed by viruses in their sojourn in the host after introduction by natural or experimental channels. These tropisms when sanely interpreted throw a flood of light on the potentialities of viruses for quite remarkable and specific adaptations and they are probably the strongest argument for the view that viruses are essentially living entities.

I propose to take first the case of vaccinia as the type of a virus whose affinities are almost solely towards cells of the mesoderm. Some years ago I devoted a good deal of time and research to this subject and I should like to refer to the main outcome of that work. The instigation to it was the claim of practically all the older workers on the pathology of variola (a claim advanced again by Levaditi and Nicolau in 1923) that the virus of vaccinia possesses a definite affinity for epiblastic structures wherever situated. Levaditi and Nicolau were of opinion that proliferative changes in epithelial cells set up by epilation or other local irritation favoured the fixation of virus which required such active and mitotic cells in which to multiply. A pre-eminent affinity of vaccine virus for epiblast did not seem to me to be consonant with the fact that immunity to vaccinia is of a general nature, as evidenced by the discovery of antiviral or virus-neutralising bodies in the blood of recovered animals by Sternberg. I decided,

therefore, to submit this question of alleged epidermotropism to experimental and histological investigation on the rabbit. This work involved the periodical removal from the same animal of areas of skin at different dates after the introduction of the virus either by intradermal inoculation or by skin scarification. The lesions at the earliest dates after introduction of the virus showed clearly that the vascular tissue in the dermis, including the endothelia of small vessels and the resting histiocytes, were the first structures to be attacked and that later proliferative changes in the local reticulo-endothelial system took the chief part in the process of repair and localisation of the lesion. Implication of the epidermal cells of the Malpighian layer and of the hair follicles was often apparent enough though not a necessary accompaniment of changes in the depth. It is true that in these circumstances there is a definite tendency on the part of the proliferative basal layer of epithelium to go on proliferating, giving rise sometimes to irregular epithelial outgrowths, but such happenings are well known to occur when a variety of foreign bodies, India ink for example, are injected into the dermis causing a local polynuclear or mononuclear infiltrate which has to be got rid of through a break in the surface epithelium as a slough. I have taken very much the same view of what happens in the comb of a hen which has been scarified with fowlpox. Here, as you are aware, the microscopic section of the lesion undoubtedly presents an appearance suggestive of very extensive and irregular production of epithelial elements in addition to the very considerable dermal disturbance below. These epithelial cells in the much thickened and distorted Malpighian layers are enormously swollen and ballooned, and contain in their cytoplasm the well known Bollinger bodies. I have not been able to satisfy myself that the virus of fowlpox has as its main and characteristic effect the proliferation of epithelium. Owing to the enormous exudate passing up from the dermis and collecting under the very dense corneum of the hen's comb, the normal removal of effete epithelium is interfered with, while the basal layer retains its proliferative power. Hence the picture which so closely simulates at first sight an epithelioma and which indeed gave rise to the old term for fowlpox—epithelioma contagiosum.

It was possible to show also that direct introduction of vaccinia virus into the peritoneum, the spleen and the subcutaneous tissue led

to characteristic lesions in the reticulo-endothelial tissue in these regions, the primary effect being the production of a karyorrhectic focus with accompanying necrosis of capillary endothelium and often haemorrhage followed by enormous proliferation of reticulo-endothelial tissue surrounding the lesion. Such proliferation determines the extrusion of the slough and forms the material from which a new vascular apparatus and collagen develop.

In a further experimental study of this question of the mode of attack of vaccine virus when introduced into the system, the problem was attacked by ascertaining the influence on the behaviour of virus exerted by simultaneous injection of India ink, and also the effect of superposition of virus on sites previously infiltrated with India ink. It emerged clearly from this work that the intracutaneous response to vaccinia in rabbits could be completely modified in degree and character or even completely abolished if ink was introduced in mixture with virus, while superposition of virus at different dates after infiltration of dermal spots with India ink led to complete abeyance of the usual response. Analysis of the phenomenon showed that the absence of response could not be attributed to any direct action of ink on virus in the direction of rendering it inert but was in all probability due to the enhancement of local defense mechanisms by stimulation of the local reticulo-endothelial tissue following the injection of the ink. The results have a very definite bearing on phenomena associated with absence of response to viruses and bacteria when introduced into areas which have been at some previous date the seat of inflammatory reaction. Also in cross-immunity experiments with viruses when skin sites are employed, it is essential to choose sites not previously used or sites in which no residual inflammatory reaction persists. While the attack of viruses when introduced into skin by scarification or intradermal injection appears to be primarily on the reticulo-endothelial structures and can be profoundly modified by any interference with those structures, we are not yet in a position to answer the question why the viruses of variola and other exanthemata have a definite predilection for skin as apparently the main site of their proliferation, after their transport via the blood stream from almost certainly an initial pulmonary focus. I have said 'only apparently' their main site of pullulation, for we have learnt a good deal in recent

years of the potentialities of differently passaged strains of virus for generalisation throughout the body as well as the skin. What would seem at first sight so slight a difference as passage from surface skin to surface skin and from dermis to dermis may have a profound effect on the intensity of the resulting lesion. For example, a strain of neuro-testicular lymph which I succeeded in adapting to the dermis of the rabbit by successive intradermal passage, remained almost powerless to produce characteristic vesiculation on the shaved skin, while a strain of ordinary calf lymph which I succeeded in adapting to the dermis of the rabbit, though with some initial difficulties, practically lost the property it had initially of producing the ordinary picture following scarification. Differences such as these between strains may perhaps indicate modifications in the metabolism of the virus determined by the oxidation-reduction potentials met with at different levels of the skin corresponding with the initial site of implantation. Possibly the inability of the dermis-passaged strain to vegetate typically on the scarified skin may be due to its having become accommodated to more anaerobic conditions in the depth of the dermis, but the matter is one for further investigation.

It has been shown by experiment that vaccinia virus can be recovered from the blood, from the tonsils and nasopharynx after ordinary cutaneous vaccination. It has not, however, been recovered from the cerebro-spinal fluid after ordinary vaccination though it has in some cases been demonstrated in this situation when there has been some concomitant disturbance in this region as, for example, of tuberculous meningitis. The further demonstration that strains of vaccinia can be obtained by visceral passage (usually through brain and testicle) which have the property of producing lesions in organs after introduction at any site, finally rendered the postulate of direct epidermotropism of this virus irrelevant. The mechanism of this generalisation is not easy to explain. It may be due to a real enhancement of virulence of the actual virus bodies or only to an increase in the mere numbers of those agents in unit volume of the tissue in which they have proliferated. This question may be answered soon by direct comparison of the washed elementary bodies recovered from dermal or visceral lesions in potency test. Whether the strain of vaccinia used is of dermal or visceral origin, there is no good evidence that it can pass

the blood brain barrier. Only when vaccinia virus is directly inoculated into the brain can it set up disturbance which takes the form of a meningitis. It is acting in the brain, just as elsewhere, only on the available mesodermal elements. There is no primary neurotropism. This absence of experimental evidence of true neurotropism of vaccinia virus is one of the main arguments against the view that postvaccinal encephalitis is simply a manifestation of vaccinia virus. Apart from the meningitis which vaccinia undoubtedly produces when introduced into the brain, there is nothing of the nature of the perivascular demyelinization process which is held to be pathognomonic for these post-infectional encephalomyelitic phenomena.

Till further notice I take the view that postvaccinal encephalitis is more probably the result of activation of some latent virus parasitising certain hosts and that it is on all fours with the similar syndromes following occasionally measles, chickenpox, etc. Its solution, however, is an urgent problem. It would seem to be bound up with the elucidation of those spontaneous encephalomyelitic syndromes associated with demyelination processes which do not necessarily follow some precedent infection. Though clinical nervous conditions in animals (dogs) have been found associated with demyelination phenomena, they have not so far been capable of experimental transmission. Some recently studied new types of encephalitis in man, such as the St. Louis type and the highly interesting ascending myelitis type due to what is now called the B. virus, present nothing of the nature of perivascular demyelination. The phenomenon must await further research. It may indeed have some obscure metabolic origin, the activation perhaps in certain predisposed persons of some lipolytic ferment. The fact that cases of postvaccinal encephalitis have been noted not infrequently in members of the same family and in twins would point perhaps to some special family predisposition of a metabolic nature.

I come now to consider the phenomenon of neurotropism exhibited by a group of viruses whether such behaviour is the normal attitude of the virus or only one for which some particular adaptation has been necessary. During recent years this subject has received much attention and some surprising results have been obtained from the study of adaptations dictated largely by trial and error. No organ is omit-

ted now in which to test the infective power of a virus seeding and least of all the brain and testicle. Such is the modern trend of virus work and it must be admitted that much of scientific interest and practical value has emerged since the original demonstration by Pasteur that the street virus of rabies could, by repeated passage through the brains of rabbits, be converted to a strain which was no longer capable of producing rabies after inoculation into a skin site, but which exhibited the power of inciting an active immunity response in the blood. At the Lister Institute my colleagues Hurst and Fairbrother undertook a few years ago a renewed study of the mode of spread in the body of the virus of poliomyelitis. Louping-ill, another virus with certain neurotropic properties, has also been studied and of late the viruses of Pseudorabies and Equine encephalomyelitis have been studied by Hurst on very similar lines to those of poliomyelitis, first at Princeton and subsequently at the Lister Institute. Another virus, the B. virus, of great human interest, has also, as I have said, been studied by Sabin during the past year at the Lister Institute and some extraordinarily interesting findings have emerged from his work to which I shall refer presently.

Neurotropism should indicate a direct primary attack on the neuron by a virus. This attack may assume various forms, acute or otherwise, but the typical attack on the neuron often progresses to necrosis of the cell. It used to be the current belief that in poliomyelitis the primary attack (in man at any rate) was on the vascular tissue in the cord and that the implication of the nerve cells in the neighbourhood was a secondary phenomenon. Now there would appear to be little doubt that the attack on the nerve cell is primary and the vascular and cellular reaction is simply a local response to the presence of necrosis in the neighbourhood. With highly virulent strains of poliomyelitis in the monkey this vascular response may be entirely in abeyance as also in acute Pseudorabies infections in the rabbit where the animal may die without any further developments.

The attack on and the destruction of a nerve cell in the cord is followed normally by migration of polynuclears from the adjoining vessels. The dead cells are dealt with in the first instance by the polynuclears, and later the microglial leucocytes, the homologues of the histiocytes of connective tissue, come along and carry away the

effete polynuclears and nerve-cell residua. The sequence of events is precisely that which occurs when, *e.g.*, the vaccinia virus is inoculated into the dermis. Cellular response on the part of the adjoining tissue is usually greater, the less acute the primary attack on the neurons. This we see clearly when we compare the histological pictures in human and in experimental monkey poliomyelitis.

Typical acute necrosis of neurons such as we see in rabies and poliomyelitis occur also in louping-ill, herpeto-encephalitis in the rabbit (we know of no such thing as herpetic encephalitis in man), yellow fever, pseudorabies, virus III and the B. virus.

Along with this acute necrosis of the neuron there may be as well other forms of less obvious attack, *e.g.*, in the cornu ammonis in rabies where the neurons containing in their cytoplasm the Negri bodies appear little altered.

Before the attacked cell progresses to necrosis there are usually to be seen peculiar changes in the nucleus leading to the development of the intranuclear inclusion and it would appear from recent work on pseudorabies and equine encephalitis, to which I shall shortly refer, that the early changes in the nucleus which lead up to the full inclusion are really the earliest signs of attack of virus. They certainly support the view that the virus penetrates directly to the nucleus of the neuron and ultimately destroys it with the result that the whole cell becomes a shadow. With regard to the secondary reaction in vascular structures when present, it may be difficult to say how far it is simply a reaction to the damaged neurons in the vicinity and how far it may be due to some concomitant attack of virus. It is now clear, however, from study of these concomitant meningitic disturbances that in certain virus diseases the virus may attack both neurons and structures of mesodermal origin. This double affinity in the case of certain viruses has been clearly elucidated by the fact that inclusions of similar type to those in the neurons appear also in cells of the vessels and meninges. We have, therefore, two main groups of viruses having neurotropic tendencies (1) which strike mainly at neurons and (2) which also mainly strike the neurons but which may or may not parasitise mesodermal cells as well.

The first group would be represented by rabies and Borna's disease, which have no mesodermal affinities. They are the strict neuro-

tropes. The second group would contain pseudorabies, herpes (in the rabbit) and virus III, which can excite lesions in many organs and directly parasitise their cells.

Possibly yellow fever and salivary gland virus and lymphogranuloma inguinale form a group by themselves in that their adaptation to brain is a purely experimental adaptation and so far as we know in the case of yellow fever has no counterpart whatever in the clinical syndrome of that disease. It is a genuine adaptation comparable with the change incurred by street rabies virus after passage through rabbit brain.

Now if there are these distinctions in cell tropisms they should be reflected in the mode of spread after introduction into the body by various routes.

The evidence that the virus of rabies takes the nervous path to the central nervous system dates back to the demonstration that section of the nerves supplying the area into which the virus is introduced prevented the development of rabies. Of late, discussion has centred on the precise route taken by the virus, *i.e.*, whether by the perineural lymphatics, which would bring the virus to the cerebrospinal fluid or by the axis cylinder, which would lead directly to the neuron. It was at one time thought that poliomyelitis traversed the perineural lymphatics and so reached the cerebrospinal fluid, which then acted as the disseminating agent, but this view is quite discordant with the fact that the virus has never been recovered from the cerebrospinal fluid during the incubation period in man and it is quite exceptional to recover it from the cerebrospinal fluid in the monkey during this period.

Hurst and Fairbrother failed to get during the incubation period the poliomyelitis virus in the cerebrospinal fluid after introduction into the sciatic nerve. It is unlikely, therefore, that the route followed is normally the perineural lymphatic one and there is now growing evidence that the nerve fibres themselves—the axis cylinders—form the path to the central nervous system. There is no real difficulty in imagining the possibilities of a route of this kind seeing that penetration of an axis cylinder really means penetration of an extension of the neuron. The ultimate disposition of the penetrating virus will depend on whether it has penetrated a sensory fibre, in

which case lesions will appear in due course in the spinal ganglion, or whether it penetrates a motor fibre, in which case the anterior horn cells, or the motor nuclei in the medulla, will exhibit the first symptoms of attack. Goodpasture and Teague, in their work on herpes, showed that when this virus was injected into the sensory division of the 5th. nerve lesions appeared at a point immediately central to the plane where the sheath of Schwann disappears, thus causing an escape of virus into the susceptible tissue of the Gasserian ganglion. When inoculated into the motor division, however, the first lesions were to be found in the motor ganglion cells of the 5th. nerve nucleus. Herpes perhaps takes both routes.

In experimental poliomyelitis Hurst and Fairbrother found that after inoculation of the virus into the left sciatic nerve, the virus appears first in the lumbar cord and soon afterwards in the leg area of the right motor cortex. At this time the cervical cord, the arm area of the right motor cortex and the left motor cortex were free. Later, virus appeared in the cervical cord, the leg area of the left motor cortex and in the arm area of the motor cortex of both sides, a distribution which would be expected if the pyramidal fibres were the transmitting mechanism.

Now a word on the blood phase which certainly comes into play in those neurotropic viruses which have affinities also with mesodermal tissue—the so-called pantropic group.

Pseudorabies. With this virus the histology of the lesions differs to some considerable extent with the animal inoculated. Thus in rabbits a necrotic and inflammatory lesion occurs at the site of inoculation if subcutaneous, intradermal or intramuscular. The virus proceeds up the peripheral nerve and a close correlation exists between the time of occurrence of lesions in the corresponding spinal ganglia and the occurrence of local itching, one of the cardinal symptoms exhibited by inoculated rabbits. The virus, reaching the ganglia and cord, produces necrosis of neurons and also of glial cells and intranuclear inclusions appear in both these systems of cells and in mesodermal elements of vessels and meninges after intracerebral inoculation. Though the virus reaches the central nervous system by the nerve path, there is virus also in the blood. Indeed, when virus is inoculated intravenously, it is capable of setting up necrotic foci in

various organs such as the adrenals (*Cf.* herpes and generalising vaccinia). From these organ lesions late spread of virus takes place by the nerve path to the central nervous system. There is no evidence that when introduced into the blood stream the virus can break through the blood brain barrier.

In the monkey, nuclear inclusions occur only in neurons and glial cells, while in the pig the vascular lesions predominate, nerve cell degeneration being very slight. There is absence of cell inclusions. Thus in the monkey the virus behaves almost as a strict neurotrope.

The B. virus. This virus would appear to have a mode of spread in the body very similar to that of Pseudorabies. When inoculated intradermally an inflammatory reaction occurs with necrosis and often haemorrhage. There are no vesicles as in herpes. Even though the skin lesion may be of small dimensions, paralysis sets in inevitably round about the 8th. day and progresses upwards to a fatal issue.

About a day before paralysis appears there is a period of licking and biting of the inoculated skin zone as in pseudorabies, in all probability due to invasion of the spinal ganglia by the virus. Corneal inoculation, as in herpes, leads to a definite encephalitis and death, while intravenous inoculation causes a paralysis of all extremities but no signs of encephalitis. Necrotic foci are present in liver, spleen and adrenals and from these visceral foci the virus passes along the peripheral nerves to the cord as in pseudorabies. There is no evidence here either that the virus can pass directly the blood brain barrier. Sabin has recorded an extensive series of experiments designed to elucidate the immunological connection, if any, of the *B. virus* with Herpes simplex and Pseudorabies. The outcome of this work has been to show that the B. virus can be easily differentiated from herpes simplex by active and passive immunity tests. It was noted, however, that certain hyperimmune antiherpes sera protected guinea-pigs from minimal, but constantly infective, doses of pseudorabies.

Also certain anti-B. virus sera could protect against a minimal dose of Pseudorabies.

There would appear, therefore, to be evidence of the existence in these three viruses of some partial common antigen, a result of very considerable scientific interest for the systematic treatment of viruses on immunological lines—a subject so far that is little known.

Before I conclude may I allude to the filterable fowl tumours and their species affinities and the virus theory of their nature. An excellent review of this subject has appeared recently by C. H. Andrewes. These fowl tumours, like those of mammals, are of very diverse histological types. Could they all be caused by one virus or by many different viruses? Andrewes tried to secure evidence on this question by cross testing of the neutralising bodies that are found in fowls bearing chronic tumours or tumours undergoing regression. It was found that serum from a slow growing fibrosarcoma (M.H.1) was active against Rous No. 1 and other fibrosarcomata but not against Fujinami. Apparently some community of antigen exists in these tumours.

Certain gaps between fowl tumours and mammalian tumours may possibly be filled up when we study viruses such as the Shope fibroma and the Shope papilloma.

It is of very great interest that Rous and Beard have recently shown that autoplasts from the Shope papilloma to the internal organs of rabbits show definite characters of malignancy.

To conclude, I think it will be agreed that the experimental study of viruses on the lines I have indicated in this lecture has proved extremely fruitful in laying bare quite unexpected potentialities for adaptation on the part of the various virus groups. Some viruses we know still prove extremely exclusive and recalcitrant and progress in their study will be to some extent retarded thereby, but there is every reason to suppose that a way out will be found leading to further advance. On this point I shall have something to say in my third lecture in connection with the actual virus agents.

HICCUP CRISIS IN TABES DORSALIS

CHARLES METCALFE BYRNES, M.D.

Associate in Neurology, Johns Hopkins University

Submitted for publication March 15, 1935

There is no reason why the patient with locomotor ataxia, like the normal individual, might not experience an attack of hiccup from any one of the many causes to which the symptom has been attributed. When, however, the attack persists, without interruption, for more than ten days, and the history and repeated examinations fail to disclose any of the recognized causes of hiccup, inquiry might well be made as to the likelihood of its occurrence as an unusual form of tabetic crisis.

W. P. H., a male, aged 51, married, was referred to me February 16, 1922, by Dr. W. H. Pearce of Baltimore, because of "shooting pains" in the arms and legs and "unsteady gait."

The family history was without significance and, with the exception of the usual diseases of childhood, the patient enjoyed excellent health until the age of 20, when he had a specific urethritis. Within the next three years there were two additional attacks of gonorrhea, but all of these infections responded to local treatment and were uncomplicated.

Twenty years ago, at the age of 31, he had a venereal sore, which was cauterized and healed without secondary manifestations, although he had received no specific treatment. With the exception of a fourth attack of gonorrhea two years later, he experienced no further ill-health until 1914, when, twelve years after the appearance of the venereal sore, there developed, on the left foot, a perforating ulcer which necessitated amputation of two of the toes. Shortly thereafter a similar ulcer appeared on the right foot and this also resulted in the loss of two toes. At this time he first experienced attacks of "lightning pains" in the arms and legs, and these attacks have recurred at variable periods and with varying degrees of intensity and duration for the past eight years. Lately, there have been only short intervals in which he was entirely free of pain and he has had, in addition, girdle sensations and pains about the lower thorax. The gait has, also, become unsteady, especially when walking in the dark, and constipation, with alternating attacks of diarrhea, gaseous eructations, and abdominal distension have been added to the symptoms. There has been no nausea, vomiting, or painful gastric attacks, and sphincter control and sexual power remain unaffected.

Notwithstanding these ear-marks of the tabetic, the disorder had not been recognized; no laboratory studies had been made; and the patient had been treated for "neuritis" and "rheumatism" until two weeks before I saw him. At about that time he first consulted Dr. Pearce, who promptly identified the disorder.

When I examined the patient he was in bed because of a severe attack of shooting pains," which were described in the manner characteristic of the tabetic. The left pupil was smaller than the right; both were contracted; neither reacted to light; but each responded during accommodation. The nerve heads were of good color, and no abnormality was noted in any of the other cranial nerves. The deep reflexes, motion and coördination in the upper extremities were unaffected. The knee jerk and the Achilles jerk were not obtained on either side; the muscles of both lower extremities were hypotonic; the heel-tibial test showed marked incoördination on each side, and postural sense was lost in the remaining toes of each foot. The gait was unsteady, and the unsteadiness was increased when the patient walked with his eyes closed. Station with the feet together was also unsteady, and this, likewise, was augmented to a marked degree when the eyes were closed. Irregular areas of hypesthesia and hypalgesia were noted on the dorsum of each foot, and these sensory changes were more extensive about the lower thoracic wall and in the upper extremities. The blood Wassermann test, previously made at the request of Dr. Pearce, was reported as negative.

February 20, 1922. The patient was admitted to the Church Home and Infirmary for further study and treatment. Upon admission he was given 0.6 gm. sodium diarsenol intravenously, and shortly thereafter he complained of slight nausea, gaseous eructation, and abdominal distension; but there was no vomiting or rise of temperature. Lumbar puncture then revealed a clear, colorless spinal fluid; pressure 230 mm. (water); lymphocytes, 20 per c.mm.; globulin, +++; Wassermann, +++. The blood Wassermann reaction was negative with two of the antigens and ++++ with the cholesterinized antigen. Direct intradural therapy was accordingly recommended.

February 21, 1922. A subdural dose of mercurialized serum containing $\frac{1}{50}$ grain of mercuric chloride was injected. This was followed by the customary reaction, characterized by shooting pains in the lower extremities and a slight rise of temperature, in this instance to 101°F.; but about six hours later, a symptom developed which I had not hitherto observed in the treatment of neurosyphilis. The patient began to hiccup. At first, the attack was no different from that occurring in the normal individual following the ingestion of irritating food or drink, although the patient had received only a mild liquid, non-stimulating diet during the preceding twenty-four hours. The hiccup continued throughout the remainder of the day and during the night without cessation, and at the rate of from ten to fifteen "hics" per minute.

February 22, 1922. The temperature had now become normal and the pain in the legs had almost disappeared, but the hiccup continued at an increased rate of about 30 per minute. Inasmuch as the reaction to the intradural treat-

ment had largely subsided and soft diet could now be permitted, the patient was discharged from the hospital the following day with my assurance that the hiccup, which still persisted, would cease spontaneously. I was then of the opinion that it was entirely of gastric origin and represented an unusual but delayed form of reaction to the sodium diarsenol.

February 26, 1922. Dr. Pearce informed me that the hiccup continued, night and day without interruption, and at the rate of about 50 "hics" per minute. This report discredited my belief that this hiccup was a delayed reaction to the sodium diarsenol, and that it might cease spontaneously.

More than two hundred "remedies" have been recommended for the treatment of hiccup. It seemed that the efficacy of some of these might now be tested. Consequently, 20 grains of zinc sulphate were given by mouth and $\frac{1}{6}$ grain of apomorphine hydrochloride hypodermically. This produced the desired vomiting, and the hiccup was relieved for about half an hour, when it recurred and persisted, with increasing rate, up to as much as 80 "hics" per minute. Oftentimes, however, the "hic" was absent; the diaphragmatic contractions were then less conspicuous and replaced by clonic wave-like movements of the abdominal muscles.

Notwithstanding the persistence of the hiccup, the following day 0.9 gm. sodium diarsenol was given intravenously. There was no unpleasant reaction to the drug, nor did it have any effect upon the hiccup, which continued without interruption at the rate of 80 per minute. Firm pressure upon the phrenic nerves, and electrical stimulation of these nerves, the epigastrium, and the abdominal muscles had no effect upon the hiccup. Likewise, repeated gastric lavage and, because it was now impossible for the patient to take nourishment by mouth, feeding through the duodenal tube and the administration of glucose by rectum failed to relieve the symptom. Traction upon the tongue, and mechanical stretching and relaxation of the diaphragm as practised by Hermann (1) were also ineffectual; and, finally, under chloroform narcosis the hiccup continued without abatement.

The gastric origin of the attack seemed now untenable and, inasmuch as the hiccup occurred shortly after the intradural administration of mercurialized serum, it was thought that this might have reflexly initiated the symptom although no plausible explanation for the reaction then suggested itself. Consequently, on March 1st, lumbar drainage of the spinal fluid was done without procuring any relief from the hiccup, although Guillain and Alajouanine (2) have since recorded an instance in which this procedure apparently relieved, for 24 hours, a prolonged attack of the epidemic type.

Although the spinal fluid, at the time of the lumbar drainage, was clear, colorless, and contained only 15 lymphocytes per c.mm., the respiratory embarrassment, inadequate nourishment, increasing exhaustion, and a dull, boring epigastric pain created a situation in which relief seemed imperative. Radical measures, such as injection or section of the phrenic nerves, were given serious consideration, but it seemed unlikely that interruption of the phrenic impulse

would relieve the spasmodic contractions of the intercostal and abdominal muscles. Inasmuch as these contractions appeared at times to be quite independent of the diaphragmatic movements, particularly during the "hicless" phase of the hiccup, further consideration of operative interference was deferred.

Furthermore, interruption of the phrenic impulse is not always successful in relieving hiccup. Kappis (3) found that bilateral phrenic section in a patient who had suffered from periodic hiccup for three years did not secure the slightest relief, and Goetz (4), after the same procedure, secured relief for only two hours in a case of post-operative hiccup. Kroh (5), also, was unsuccessful in relieving the symptom by interruption of the phrenic impulse and found, furthermore, that bilateral section of the phrenic in dogs failed to produce immediate complete paralysis of the diaphragm. When, however, the phrenic nerve alone is the source of irritation the operation should be successful, and instances are recorded in which hiccup has been relieved by this means.

Although Symes (6) states that Romberg had shown that stimulation of the phrenic does not produce hiccup, the general opinion that the symptom is largely due to direct or reflex phrenic irritation invited inquiry into the implication of this nerve in the tabetic lesion. Syphilitic phrenic neuritis and neuralgia have been described, but involvement of the nerve in tabes is almost unknown. Levy-Valensi (7) has reported a case of tabetic phrenic neuralgia, but hiccup was not a symptom, and I have found no record of a phrenic neuritis accompanied by hiccup. With so little evidence that the hiccup in my patient was actually due to disease of the phrenic nerve and so little assurance that interruption of its impulse would relieve the symptom, operation was not recommended.

Notwithstanding the obscurity of the mechanism of the hiccup in my patient there was much in his clinical record to suggest its tabetic origin. The recurrent gastro-intestinal disturbances, the girdle sensations and the sensory changes about the thoracic wall, the occurrence of the hiccup quite independent of the ingestion of food or any of the usual causes to which it is attributed, its prolonged duration, variability in rate, and the implication of many muscles involved in the act of vomiting presented a striking analogy to the tabetic gastric crisis.

The literature, at this time, contained little information concerning the occurrence of hiccup crisis in tabes. Gowers (8) states: "I have known frequent hiccup to accompany the vomiting" of gastric crises, and Oppenheim (9) and Karl Schaffer (10) refer to an article by Stembo who observed the symptom in two tabetic patients. More modern texts have little more to say. Sir James Purves-Stewart (11) refers to the occurrence of "diaphragmatic crisis with hiccup," and Jelliffe and White (12) mention "Laryngeal and diaphragmatic crises with cyanosis and dyspnea, pharyngeal crises with obstinate hiccup."

My previous experience in the treatment of tabetic gastric crises in which I have, upon several occasions, presumably stopped the attack by the administration of mercurialized serum led me to believe that the hiccup crisis, which I regarded as of tabetic origin and independent of direct phrenic involvement, might also

respond to this direct form of therapy. Consequently it was decided that if the hiccup had not ceased within the next 24 hours the administration of mercurialized serum was to be repeated, notwithstanding the suspicion that the initial dose might have initiated the attack.

Thus, on March 2, 1922, the hiccup still persisting at the rate of about 80 per minute, $\frac{1}{5^{0}}$ grain of mercurialized serum was given subdurally at 10:20 p.m. The hiccup stopped before the needle was withdrawn, and the patient complained immediately of pain in the lower extremities and abdomen as high as the costal margin. After two hours of relief the hiccup recurred periodically at about half-hour intervals throughout the remainder of the night; but the abdominal contractions had ceased entirely and the "hics" were feeble and reduced in rate to about 20 per minute. The following day there was an entirely free interval of about eight hours, when feeble attacks, of short duration and separated by intervals of several hours, made their appearance; but on the second day following the treatment, March 4th, the hiccup ceased entirely.

Further intravenous and intramuscular treatments were given without ill effect, and the patient continued to improve clinically. Laboratory examination May 25, 1922, revealed a clear spinal fluid, pressure, 110; cell count, 10; globulin, 0; Wassermann, 0; Blood Wassermann, 0; and no further treatment was administered until February 3, 1923. The spinal fluid obtained at this time was clear; the pressure, 150; cell count, 5; globulin, 0; Wassermann doubtful. Twelve intramuscular injections of mercury salicylate were then given at weekly intervals. April 10, 1923, because of slight return of pain in the legs and about the waist, sodium diarsenol 0.6 gm. was given intravenously. The reaction to this consisted of nausea, vomiting and diarrhea.

The following day, a third subdural dose of mercurialized serum was administered. Shortly thereafter, the hiccup recurred and continued periodically, at 20 to 30 minute intervals, for about six hours, but the contractions were feeble and of short duration. After that time there was no return of the hiccup, the general condition was improved; the pains ceased; the gait was more steady, and this degree of improvement persisted until the patient's death in 1925, from an intercurrent infection.

The first recorded instance of hiccup crisis in tabes is by Daunic (13) who described the symptom as an early pre-ataxic sign of tabes. His patient suffered at variable intervals from recurrent attacks of hiccup and epigastric pain for a period of ten years, during which time there were none of the usual signs of tabes nor did he complain of any symptoms common to the disease. Later, gastric crises, lightning pains, and ataxia made their appearance, and the hiccup and gastric crises then occurred together in attacks varying in duration from one to several hours. The hiccup, like the gastric crisis, oc-

curred without any relation to the ingestion of food and after continuing for several hours was accompanied by pain in the epigastrium and attacks of syncope. Daunic regarded the symptom as analogous to the laryngeal and gastric crises and designated it as *ictus diaphragmatique*, dependent upon an inflammatory lesion of either the phrenic, the vagus, the spinal accessory or perhaps of all three nerves. All forms of medication were ineffectual, but pressure upon the phrenic nerves often aborted an attack.

In reporting his two cases of hiccup in tabes, Stembo (14) makes no reference to this earlier paper by Daunic, and states that the condition had not been previously noted as a symptom of the disease. In one of his patients the attacks, often accompanied by pain in the epigastrium and about the thoracic wall, occurred day and night and during sleep for a period of three weeks, but there were variable intervals in which they temporarily ceased spontaneously, or after automatic or induced vomiting.

The hiccup, in his second case, first appeared two years after the primary lesion, and persisted with remissions for about 10 days. In the beginning induced vomiting frequently, but not always, gave temporary relief, and during the course of the affection spontaneous vomiting and violent eructations became added features; induced vomiting then ceased to be an effective therapeutic measure. The hiccup varied in rate from 80 to 100 per minute and, as in the first case, was associated with pain in the epigastrium and lower thoracic wall, about which there was a hyperesthetic zone on each side. Very often the patient became cyanotic and eructated large amounts of mucus; and when successful in swallowing food, experienced intensified epigastric and thoracic pain and more violent hiccup. Gastric lavage frequently procured relief for several hours and, after adoption of the complete gastric ulcer cure for one week, the attacks ceased. Inasmuch as they began and behaved like other forms of crises, Stembo was of the opinion that they were of tabetic origin, but he was unable to decide whether they were due to direct irritation of the diaphragm, the phrenic nerve, or to reflex irritation of the respiratory center.

Through the kindness of Dr. Beverley R. Tucker, of Richmond, Va., I am permitted to report, from his private records, an additional case of what appeared to be a hiccup crisis in tabes. His patient gave a

history of having had a venereal sore ten years previously, and of having suffered from persistent hiccup, unaccompanied by pain or vomiting, for a period of ten days. The signs and symptoms of tabes were classical and notwithstanding a persistently negative serology in both the blood and spinal fluid, the clinical diagnosis of tabes dorsalis was, in Dr. Tucker's opinion, unquestionable. The hiccup was apparently relieved by the administration of musk every four hours and, so far as Dr. Tucker was informed, there had been no recurrence during a period of eight years.

With this clinical confirmation from the literature of hiccup crises in tabes, I felt more secure in my opinion that the hiccup in my patient was of this nature; that it was analogous to the gastric crisis; and that I had induced it by the administration of mercurialized serum. It had not been an uncommon experience in the treatment of tabes with gastric crises to observe that either intravenous or subdural therapy, during a free interval, appeared to initiate a gastric attack as well as other quiescent symptoms from which the patient might have suffered. On the other hand, paradoxical as it may seem, I have repeatedly aborted a gastric crisis by the use of mercurialized serum during the course of the attack and, although I had formulated an hypothesis concerning the mechanism through which direct therapy might exert its effect upon the gastric crisis in one subject to the attacks, it was difficult at first to conceive of the manner in which it could have any effect upon the hiccup crisis.

In my patient, the hiccup implicated not only the diaphragm but the accessory intercostal and abdominal muscles concerned in the act of vomiting, and differed from the mechanism of vomiting only in the character of these muscular contractions. Notwithstanding the general belief in the reflex origin of hiccup through vagus, laryngeal, phrenic irritation, the symptom, even in its simplest form, does not occur independently of associated movements in the lower intercostal and abdominal muscles. Following intradural therapy it is customary to keep the patient on his back with the neck and head slightly elevated, so that the pain and sensory disorders accompanying the reaction rarely extend above the lower costal margin. It seemed unlikely, therefore, that the medicated serum, administered subdurally in the lumbar region, could have ascended to the radicular origin of the

phrenic, vagus, or accessory nerves in sufficient concentration to initiate abnormal impulses in any of these nerve trunks. It is, moreover, unlikely that, had the serum diffused into the cervical region, it would have irritated the motor roots alone; and there was no evidence that the sensory component of any of these nerves had been affected. Still, the serum appeared to have initiated the hiccup crisis.

It was necessary, therefore, to inquire into the disposition of the lower thoracic nerves in relation to the diaphragm. According to Cavalié (15), it had been suggested as early as 1862, by Luschka, that the intercostal nerves take part in the motor innervation of the diaphragm. These, he designated as "intercostal phrenic nerves," and stated that they protect the diaphragm from complete motor paralysis when deprived of its phrenic innervation; but it was also noted that they furnish additional pathways of excitation. Cavalié confirmed this statement in the anatomical study of six diaphragms, and concluded that the last six intercostal nerves, and especially the 7th, 8th and 11th, contribute regularly to the innervation of the muscle along its costal attachments; but he made no distinction in the disposition of their sensory and motor components. Sympathetic fibres, accompanying the lower intercostal vessels, also terminated in the muscle.

Careful embryological studies of the diaphragm and its innervation have been made by Felix (16) in nine specimens of human embryos. He found that the phrenic nerves furnish no branches to the pericardium, costal pleura, or mediastinal pleura, and that they innervate the pleural and peritoneal coverings of the diaphragm only in its central portion. The sympathetic fibres, situated at the periphery of the nerve, are derived from the cervical anastomotic plexus, the lower cervical and first thoracic nerves, and through connections with the sympathetic fibres of the diaphragmatic or phrenic plexus communications are established with the coeliac plexus, the ganglion phrenicum and the paraganglia. The lateral portions of the pleural and peritoneal coverings of the diaphragm derive their sensory fibres from the last five or six intercostal nerves (fig. 1); and sympathetic nerves are, as Cavalié maintained, received from the lower intercostal blood vessels.

Although the phrenic nerve furnishes the principal motor innervation of the diaphragm, its parietal and lumbar portions are innervated

almost entirely by the sympathetic and last thoracic nerves. The sympathetic component, he thinks, furnishes not only tone fibres to the diaphragm but motor fibres also. It is of interest that Felix does not accept the occurrence of a phrenic neuralgia with pain in the course of the nerve and diaphragm, but regards a diaphragmatic neuralgia as of intercostal origin. Hiccup, he maintains, is due to irritation of the diaphragmatic sympathetic plexus.

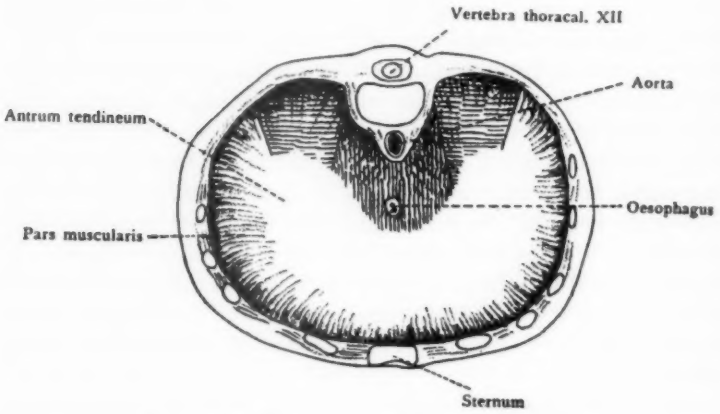

FIG. 1. THE DIAPHRAGMATIC MOTOR INNERVATION

The horizontal marking indicates the 12th thoracic innervation; the perpendicular, the phrenic and sympathetic; the unshaded area, the phrenic innervation alone. (After Felix.)

Clinical confirmation of these anatomical studies has been furnished by Capps and Coleman (17). Following paracentesis of the abdomen, distended by ascites or by artificially introduced air, smooth and rough tipped wires were introduced through the cannula. By this means various portions of the abdominal and diaphragmatic peritoneum could be stimulated. Irritation of the abdominal peritoneum produced a corresponding painful spot on the skin surface almost directly over the tip of the wire. When, however, the central portion of the diaphragmatic peritoneum was irritated, the patient complained of pain in the neck about the clavicular attachment of the trapezius muscle, but

never along the course of the phrenic nerve. As the rough tipped wire was drawn along the under surface of the diaphragm and approached its costal attachments, the pain in the neck disappeared and was replaced by spot pain on the skin surface of the lower thoracic wall.

From my clinical study and the teachings of anatomists concerning the diaphragmatic innervation, the following deductions might be considered.

The apparent spontaneous onset of hiccup in certain cases of tabes, its periodic occurrence and prolonged duration, its complete cessation when once it is relieved, and its not uncommon association with vomiting and epigastric pain supply the characteristics of a true tabetic crisis.

Inasmuch as the gastric crisis not infrequently initiates the tabetic symptom-complex, Daunic's suggestion that the hiccup crisis might also be an early, pre-ataxic sign of tabes deserves some consideration. The gastric and hiccup crises have striking analogies, and a prolonged attack of hiccup, in the absence of any discoverable cause, warrants a thorough neurological examination; and should this not disclose any of the ear-marks of tabes, serological tests of the blood and spinal fluid might well be advised.

Although diaphragmatic motor innervation is derived largely through the phrenic, a generous sensory innervation is received from the last seven thoracic nerves; the twelfth also furnishes independent motor fibres. It is not unlikely that impulses originating in these lower spinal segments might, either directly or reflexly by means of the phrenic, take part in diaphragmatic contractions. That the phrenic is not essential in the production of hiccup is found in the fact that bilateral phrenic section is not always successful in relieving the symptom. Under this circumstance it is quite possible that the irritation might be maintained through this lower spinal innervation.

Furthermore, the tabetic process has a special predilection for the thoracic cord, and it seems probable that hiccup in this disease is due to abnormal impulses originating in these lower spinal segments. It is through this mechanism that the medicated serum most likely induced the initial and subsequent attacks in my patient by irritation of a system of fibres already partly implicated in the disease. On the

other hand, persistent treatment, as in the gastric crisis, finally leads to resolution of the original tabetic process.

It is not unlikely that this lower spinal mechanism may take part in the production of post-operative hiccup, inasmuch as the peritoneum offers a wide field for the origin of impulses which might reflexly initiate abnormal diaphragmatic contractions. In discussing this phase of the subject with my friend, Dr. John Cotton, he very wisely suggested that spinal anesthesia might be effectual in relieving this type of hiccup.

In the treatment of hiccup, any form of therapy is regarded with skepticism, but failure of many of the generally recommended measures to relieve the symptom in my patient, and the promptness with which it ceased upon the intradural administration of mercurialized serum suggest that the method is of therapeutic value in the treatment of the tabetic hiccup crisis.

9 E. Biddle Street, Baltimore, Md.

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THE QUANTITATIVE SPECTROGRAPHIC ESTIMATION OF BLOOD LEAD AND ITS VALUE IN THE DIAGNOSIS OF LEAD POISONING

HAROLD BLUMBERG, Sc.D.[1] AND T. F. McNAIR SCOTT, M.R.C.P. Lond., M.R.C.S. Eng.

From the Biochemical Laboratory of the Johns Hopkins University School of Hygiene and Public Health, and from the Harriet Lane Home of the Johns Hopkins Hospital, and the Department of Pediatrics of the Johns Hopkins University School of Medicine

Submitted for publication April 17, 1935

In a previous communication by Shipley, Scott, and Blumberg (1), it was demonstrated that the spectrographic detection of appreciable amounts of lead in the blood constituted a convenient aid to the clinical diagnosis of plumbism. Following this observation, a procedure was standardized and used for the quantitative spectrographic estimation of blood lead, a brief account of which has appeared (2). This report presents the results of two years of experience with the method, which was proved to be of value in the diagnosis of 36 cases of clinical lead poisoning.

METHOD FOR ANALYSIS OF STANDARD LEAD ASHES

Preparation of standard lead ashes

In order to establish a quantitative method, it was necessary to prepare a series of ashes containing varying amounts of lead. Since the basic ash had to be lead-free, an artificial base was made so as to approximate the composition of blood ash. A mixture of NaCl, K_3PO_4, $Ca_3(PO_4)_2$, and Fe_2O_3 was used for this purpose.

A satisfactory sample of NaCl (Chemically Pure) was available, and lead-free preparations of K_3PO_4 and $Ca_3(PO_4)_2$ were secured after precipitating traces of the metal from solutions of C.P. potassium and calcium salts by means of hydrogen sulfide. The analysis of 25 C.P. iron compounds revealed that lead, like manganese, was a constant contaminant. However, samples of iron wire (for standardization)

[1] Fellow in Medicine of the National Research Council, 1934–35.

and a spectrographic iron electrode were found to be lead-free. A piece of the electrode was then dissolved in distilled nitric acid, the iron was precipitated as $Fe(OH)_3$ by an excess of distilled ammonium hydroxide, and the precipitate was converted into Fe_2O_3, a lead-free product, by heating in a muffle furnace.

The mixture of the basal ash was made from NaCl (47%), K_3PO_4 (32%), $Ca_3(PO_4)_2$ (16%), and Fe_2O_3 (5%). Standard lead ashes were made by evaporating known amounts of standard lead nitrate solutions on weighed portions of the base, after which the mixture was ground to a uniform composition. In this way a series was prepared which contained lead in the following concentrations: 0.0000, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1%.

Method

Apparatus: The instrument used was a Hilger E 1 large quartz spectrograph, operated in conjunction with a graphite arc and a quartz sphero-cylindrical concentrating lens. The electrodes were Acheson graphite rods, 6.5 mm. in diameter; the upper rod was the negative pole, and the lower rod was the positive. The current through the arc was 5 amperes, at approximately 45 volts. Eastman No. 33 photographic plates were used, and were developed in an Elon-Hydrochinone developer under standardized conditions.

The analysis: By means of a Hartmann diaphragm, 3 spectrograms are taken in juxtaposition (fig. 1). The first or upper picture is that of the empty graphite electrode, the middle is the ash on the electrode, and the lowest is a drop of a known lead solution (lead nitrate) to supply reference lines. In the taking of the upper and lowest pictures of the triplet, the arc is simply burned for approximately 20 seconds. For the middle picture, which gives the actual analysis, the lowest electrode is loaded once with the ash (approximately 8 mg.), and the arc is burned for 30–40 seconds, which is about sufficient to volatilize all of the ash. The procedure is repeated for a second load with a burn lasting 20–30 seconds, that is, long enough to make a total burning time of exactly 60 seconds.

Reading the results: The plate is read by a rapid visual inspection with a hand magnifying lens. For its quantitation the method depends upon the number and intensity of the lead lines present in the

spectrum of the ashes (fig. 1). As the concentration of lead increases, there is a corresponding increase in the number and intensity of the lead lines. This is illustrated by the following chart, which repre-

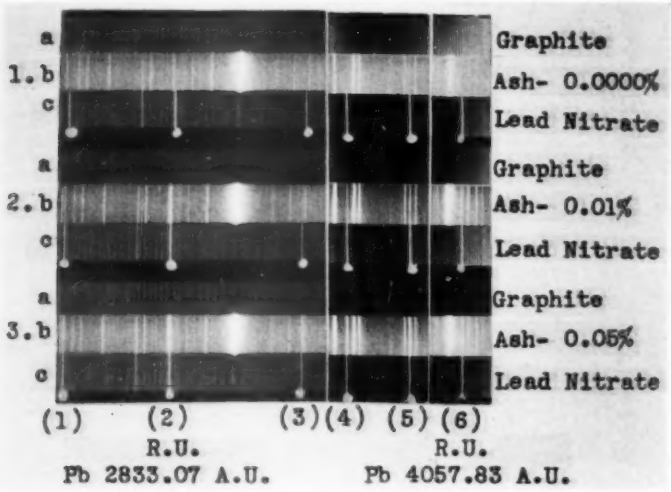

FIG. 1. STANDARD LEAD ASHES

The portions of the plate containing the essential lead lines have been selected for the condensed illustration. The dots mark the positions of the 6 principal lead lines used, which are (from left to right): (1) 2802.09, (2) 2833.07, (3) 2873.32, (4) 3639.58, (5) 3683.47, and (6) 4057.83 Angstrom Units. The lines (2) and (6), at 2833.07 A.U. and 4057.83 A.U., are the *raies ultimes*, or most persistent lines.

1. In the first spectrogram, *a* is the control spectrum of the empty graphite electrodes; *b* is the spectrum of the lead-free ash, and *c* is a drop of a lead nitrate solution to supply reference lines. Notice that none of the lead lines is present in *b*. (The faint line almost coinciding with the minor lead line (5) is due to iron.)

2. *b*, Ash containing 0.01% lead. Notice that all 6 of the lead lines are present, although (3) is very faint.

3. *b*, Ash containing 0.05% lead. Notice that all 6 of the lead lines are heavier, even (3) being easily visible.

sents readings of standard lead plates made from the artificial ashes. (It should be noted that the table is constructed from the actual negative, in which the lines are black, while the illustration is the

positive, in which the lines appear white. The finer differences in intensity are much more easily appreciated in the black lines of the negative than in the white lines of the positive. The weak lines at 3572.74 A.U. and 3739.95 A.U. are omitted from the illustration.)

TABLE I

Wavelengths in Angstrom Units

LEAD CON.-%	MG. PER 100 CC.	(1)	(2) R.U.	(3)		(4)	(5)		(6) R.U.
		2802.09	2833.07	2873.32	3572.74	3639.58	3683.47	3739.95	4057.83
0.0000	0.000	0	0	0	0	0	0	0	0
0.0005	0.005	0	1	0	0	0	0	0	1
0.001	0.01	1	2	0	0	1	1	0	2
0.005	0.05	2	3	1	0	2	2	0	3
0.01	0.1	3	4	2	0	3	3	0	4
0.02	0.2	4	5	3	1	4	4	1	5
0.05	0.5	5	6	3	2	5	6	2	6
0.1	1.0	6	7	4	3	6	6	3	7

Raies ultimes, or most persistent lines: 2833.07 A.U. and 4057.83 A.U.

Legend: 0, invisible; 1, visible only with the aid of a lens; 2, barely visible to the eye as a very faint line; 3, visible as a grey line; 4, visible as a thin black line; 5, visible as a slightly thickened black line; 6, visible as a moderately thick black line; and 7, visible as a very thick black line, with slight fogging.

With the large model spectrograph, it was found convenient to estimate the lead content of the ash from the readings of all the 8 different lines shown in the chart. The resulting value was thus an average of 8 different readings, and was a safequard against the possible intensity variation or the false reading of any one line. In order to find the lead content of the blood, the lead concentration of the ash was multiplied by the percentage ash content of the blood. In the absence of extreme blood changes, the ash content found by our procedure (to be described below) is about 1%. Therefore, the lead concentration of the blood may be calculated routinely by dividing the lead content of the ash in grams by 100, and multiplying by 1000 to convert into milligrams per 100 grams. Since the specific gravity of blood is just slightly above 1, no significant error in this case is introduced by substituting the conventional unit of mg. per 100 c.c. In effect, the decimal point is simply moved one place to the right, as shown by the chart.

The dependability of the method was demonstrated by duplicate analyses, which revealed little or no difference in the two results obtained from a given sample and indicated that the percentage error was within $\pm 50\%$ (a variation which actually is not great for very minute quantities). On each plate a spectrum of a known standard ash (0.01%) was recorded, so that any variation in the sensitivity of the individual photographic plates would be revealed through changes in the standard. In terms of absolute sensitivity, the limit of detection lay between 0.00005 and 0.0001 mg. (or 0.05 and 0.1γ) of lead. Relative to whole blood, it was found that 0.005 mg. per 100 c.c. could be detected by the standardized procedure.

APPLICATION OF METHOD TO ANALYSIS OF BLOOD

Preparation of blood ash

Five cubic centimeters of blood are withdrawn from the vein and emptied into a test tube which has been cleaned in strong nitric acid and rinsed well in distilled water. (Originally the syringes also were cleaned in nitric acid, but it was found that for routine purposes no significant error was introduced through the use of syringes boiled in the usual fashion, although a rinsing in distilled water is an advisable precaution.) One and a half cubic centimeters of distilled sulphuric acid are added to the blood in a silica ashing dish and the sample is digested and charred on a hot plate. Ashing is completed in 8 hours or less in an electric muffle furnace at a temperature not exceeding 500°C. The resultant ash is weighed (or routinely may be assumed to be 1%), powdered and triturated in an agate mortar, and is then ready to be examined spectrographically.

Method

The analysis: The ash is burned in the electric arc exactly according to the standardized procedure just described for the preparation of the standard lead plates. Each blood sample is analyzed twice (there is ample ash for two analyses) in order to check the result.

A 4 x 10 inch photographic plate may be used conveniently for 8 analyses, or for checked results on 4 samples. When the analyses are finished, the plate is developed, fixed, and washed. If time is an important factor, the result may be obtained while the plate is still

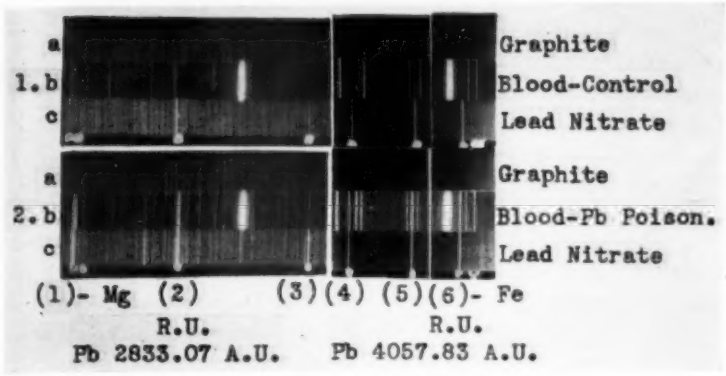

FIG. 2. BLOOD ASHES

The portions of the plate containing the essential lead lines have been selected for the condensed illustration. Dots mark the positions of the 6 principal lead lines used, which are (from left to right): (1) 2802.09, (2) 2833.07, (3) 2873.32, (4) 3639.58, (5) 3683.47, and (6) 4057.83 Angstrom Units. The lines (2) and (6), at 2833.07 A.U. and 4057.83 A.U. are the *raies ultimes*, or most persistent lines. To the right of (6) are the two comparison iron lines, Fe 4063.76 A.U. and Fe 4071.90 A.U. (marked by double dot). Slightly to the right of (1) is the comparison magnesium line Mg 2802.71 A.U. (marked by a dot). The heavy lines slightly to the left of (6) are due to potassium; the heavy lines (actually two lines) between (2) and (3) are due to magnesium and sodium.

1. In the first spectrogram, *a* is the control spectrum of the empty graphite electrodes; *b* is the control blood ash, showing a lead content of 0.01 mg. per 100 c.c.; and *c* is a drop of a lead nitrate solution to supply reference lines. Notice that only the lead *raies ultimes*, (2) and (6), are visible, and they are faint (the faint line almost coinciding with the minor lead line (5) is due to iron). Notice also that the comparison iron lines are much heavier than the lead line (6), which is Pb 4057.83 A.U.; furthermore, the comparison magnesium line at 2802.71 A.U. is fairly heavy, while the lead line (1), which is Pb 2802.09 A.U., is not even visible.

2. *b*, Blood ash from case of lead poisoning, showing a lead content of 0.5 mg. per 100 c.c. Notice that most of the lead lines are heavy, and all 6 are easily visible. Notice also that the lead line (6) is heavier than the comparison iron lines, and the lead line (1) is just as heavy as the comparison magnesium line Mg 2802.71 A.U.

wet; in general, however, the plate is allowed to dry before examination over a lantern box.

Reading the results: The intensities of the lead lines in the blood spectrum are read by rapid visual inspection, as described before. The lead content of the blood is then estimated by comparison with the chart of the standard lead plates. By the procedure described, the result of the analysis may be known in 12 hours or less, chiefly dependent upon the time required for ashing.

ANALYTICAL RESULTS AND ESTABLISHMENT OF BLOOD-LEAD VALUES

During the course of this investigation analyses were made on more than 300 samples of blood, which included specimens from 36 cases of clinical lead poisoning, as well as several cases of asymptomatic lead exposure. The majority of the patients with plumbism were children in Dr. Park's clinic at the Harriet Lane Home of the Johns Hopkins Hospital, but the series also contained cases from other departments and hospitals. Of the 36 patients, 6 were adults who had contracted lead poisoning from their occupational pursuits. The first few cases of intoxication in children, studied in the summer and fall of 1932, were due to the burning of lead storage battery boxes, as reported by Williams *et al.* (3). In the cases occurring since that time, the poisoning apparently resulted from the ingestion of lead-containing paints by children with pica, although in several instances the source of exposure could not be ascertained.

The results of the study have consistently shown a significant increase of the blood lead in lead poisoning, so that the pathological samples may easily be distinguished from controls (fig. 2). Through the use of a longer burning time in the standardized procedure, the lead trace in the control bloods, which was previously regarded as questionable (1), was brought out definitely, although in some cases it was very faint and scarcely greater than the contamination that might result during the routine sampling of blood. From the data secured, the following values for blood lead have been established:

TABLE II

Values for blood lead

		mg. per 100 cc.
Non-pathological (clinically negative)	"Normal" lead	0.005–0.05
	Slightly abnormal exposure	0.05 –0.1
Pathological borderline (clinically questionable)	Sub-clinical or mild clinical lead poisoning	0.1 –0.2
Pathological (clinically positive)	Lead poisoning or convalescence	0.2 –1.0
Critical range for appearance of symptoms		0.1 –0.2

The values given in the table are essentially the same as those previously reported for a smaller series (2), except that the upper limit of the pathological range has been extended from 0.5 mg. to 1.0 mg. per 100 c.c.[2] This change was made as a result of the high blood-lead figures observed during the past summer. On one occasion, which was that of a rapidly fatal lead encephalopathy in a young negro boy, the blood lead reached 1.0 mg. per 100 c.c. The lead content of the majority of the control bloods was approximately 0.05 mg., while the majority of the lead poisoning samples lay in the 0.2–0.5 mg. range.

Since the primary object of the method was the detection of lead poisoning, aids were sought for the rapid interpretation of the results. Observation of the blood ash spectrograms revealed two convenient comparisons, or inner controls, which act as an additional check upon the quantitative blood lead estimation in determining whether or not a sample is pathological. The first lines examined are the lead line Pb 4057.83 A.U. and the adjacent iron lines Fe 4063.76 A.U. and Fe 4071.90 A.U. (fig. 2). In the non-pathological samples the iron lines appear more intense than the lead line. In the pathological bloods, however, the lead line appears at least as strong as the iron lines, and usually stronger. Since great differences in iron concentration may

[2] Since the preparation of this article, Wexler and Sobel (33) have reported on the spectrographic determination of lead in blood serum. They state that patients with clinical symptoms of lead poisoning showed concentrations in blood varying from less than 0.1 mg. to more than 1 mg. of lead per 100 c.c.

disturb this comparison, it must be applied with caution when one is dealing with extremely severe anemias. For practical purposes, nevertheless, the comparison may be safely used in the general run of cases because the lines of iron are "spectrographically buffered." The individual intensity of any one of the numerous iron lines varies only slightly with comparatively larger variations in iron concentration.

The second inner comparison is between the lead line Pb 2802.09 A.U. and the adjacent magnesium line Mg 2802.71 A.U. (fig. 2). In the non-pathological samples the magnesium line appears very much heavier than the lead line, whereas in the pathological samples the lead line has an increased density that makes it almost as heavy as, or slightly heavier than, the magnesium line. This comparison, of course, may be disturbed by gross changes in blood magnesium, but such interference is unlikely. By means of these two simple comparisons, the pathological bloods have been distinguished from the non-pathological ones at a glance.

COMPARISON OF SPECTROGRAPHIC AND CHEMICAL RESULTS

As a check upon the spectrographic method, simultaneous chemical and spectrographic analyses were made upon 50–100 c.c. samples of ox blood to which definite quantities of lead had been added. The analyses were carried out by a procedure similar to the Kehoe, Thamann, and Cholak modification (4) of Fairhall's s-diphenyl carbazide method (5). A comparison showed the chemical results to be lower than the spectrographic figures by approximately 0.08 mg. per 100 c.c. In view of the difficulty of recovering small amounts of lead by chemical methods, as has been pointed out by Behrens (6), the discrepancy was considered to be due to a loss of lead during the many and varied manipulations necessary in the chemical procedure. Indeed, Kehoe and coworkers (7) have just recently recognized that there is an average loss of 0.07 mg. per sample inherent in their chemical procedure, as revealed by spectrographic control, and found amounts of lead varying from 0–0.01 to 0.12–0.13 mg. per 100 c.c. in the blood of a group of medical students.

During the past few years extensive studies on the chemical evaluation of blood lead in lead poisoning have been reported by Litzner

and Weyrauch (8) (9), who, like Bass (10), consider the lead content of the blood to bear a close relationship to the appearance of clinical symptoms. The results of their chemical studies allow the following comparison to be made with our spectrographic values:

TABLE III

Blood lead values and appearance of clinical symptoms

METHOD	LITZNER AND WEYRAUCH (9)	AUTHORS
	Chemical	Spectrographic
	mg. per 100 cc.	*mg. per 100 cc.*
Clinical symptoms absent	0.01–0.04	0.005–0.1
Clinical symptoms first appear	0.04–0.07	0.1– 0.2
Clinical symptoms present	0.07–0.5	0.2– 1.0
Critical level stated	0.06	0.1– 0.2
Majority of cases	0.07–0.1	0.2– 0.5

It is thus apparent that the critical level, as determined spectrographically, is from 0.04–0.14 mg. (or an average of 0.09 mg.) higher than the critical level found by chemical investigations. This difference is consistent with the results of our previous comparison, in which a discrepancy of approximately 0.08 mg. between the two types of determination was noted. In consideration of the probable losses in the chemical procedures, it appears likely that the spectrographic figures represent the actual blood lead values.

PERSISTENCE OF HIGH BLOOD LEAD AFTER CESSATION OF EXPOSURE

In our studies it was also observed, as Litzner and Weyrauch (9) have reported in their adult cases, that clinical symptoms of plumbism disappear before the gradually decreasing blood lead falls to the nonpathological levels. Indeed, it was somewhat surprising to find that comparatively large amounts of lead continued to circulate for many months after the end of lead exposure, particularly in children. Some of the latter could be followed during and after recovery from their lead encephalopathy; the blood analyses in these cases demonstrated that 6–12 months were required for the blood lead to drop to about 0.1 mg. per 100 c.c. After falling to the pathological borderline, in

several instances the blood lead persisted at this level for as long as an additional 12 months.

This fact, of course, is quite in accord with the interesting observations of Park, Jackson, Goodwin, and Kajdi (11) in regard to the "double zone" phenomenon which is frequently seen in the x-ray plates of long bones from old cases of lead poisoning in children. With the growth of the bone, the initial lead line, which was first described by Park and coworkers (12) (13), Vogt (14), and Caffey (15), is gradually resolved into a double line. As new bone growth occurs, the lead is continually liberated into the circulation during the physiologic resorption of bone and is partially redeposited in the growing bone at the new cartilage-shaft junction. Consequently, it should be expected that the blood lead would persist at an abnormally high level throughout this process. Since the liberated lead is probably accompanied by phosphate, the protective influence of which has been demonstrated by Shelling (16), continued relapses are not usually encountered during this process. In a comparative study of the x-ray plates and blood-lead analyses over a period of more than two years, a rough correlation has been noted between the disappearance of the lead line and the return of blood lead to non-pathological ranges.

During the convalescent period the blood analysis thus often shows lead circulating in pathological amounts in the absence of outspoken clinical symptoms. In such conditions, therefore, the lead poisoning must be regarded as latent rather than as absent. Slight metabolic disturbances might cause the abrupt reappearance of severe clinical symptoms as a result of the excessive release of stored lead. The occasional recurrence of attacks in the absence of additional exposure is well-known evidence of the potential intoxication which is present during convalescence.

LEVEL OF BLOOD LEAD AND SEVERITY OF SYMPTOMS

It is evident that the values for blood lead must form a continuous series from the non-pathological traces to the pathological amounts found in outspoken plumbism. From the chemical point of view, therefore, the difference between health and lead poisoning is quantitative rather than qualitative. Although clinical symptoms cannot

be expected to parallel exactly the quantities of circulating lead because of the individual variation in biological response to injury, nevertheless there appears to be a range, established from our data as 0.1–0.2 mg. per 100 c.c. of blood, at which symptoms of lead poisoning tend to appear. This would seem to be the critical level of blood lead, as suggested by Park and coworkers (11). It seems that mild clinical symptoms may first appear at this level, although in the majority of cases with this blood-lead value the intoxication is subclinical. At this stage, no doubt, cases of borderline lead poisoning might be revealed by blood analysis before the intoxication had become sufficient to evoke clinical symptoms.

In the series of patients under observation, it appeared that the clearly pathological range, characterized by symptoms of a moderately severe or severe nature, was from 0.2–1.0 mg. per 100 c.c. The greater number of the 36 cases of clinical plumbism lay within the range of 0.2–0.5 mg. per 100 c.c. (*cf.* table III).

The blood lead values at the lower limit of the pathological range, *i.e.*, 0.1–0.2 mg., corresponded to very mild symptoms of plumbism, while the analyses approaching 1.0 mg. were in very severe cases. Between the extremes, however, there appeared to be no close correspondence between blood lead and severity of symptoms, a finding which is in accord with the observations of Litzner and Weyrauch (9). In convalescence, as mentioned previously, no correlation between the level of blood lead and outspoken clinical symptoms appears to exist.

It must be remembered that the symptomatology present at the time of examination may not be due to active lead poisoning, but may be due to the effects of permanent damage caused by acute lead poisoning months or even years before. This is particularly true of neurological symptoms, since mental deterioration, and gross signs such as hemiplegia, may be permanent sequelae of an initial lead encephalopathy and remain unaltered, whereas the level of the blood lead will tend slowly to fall to within non-pathological limits.

For purposes of diagnosis, the following statements may be made from our observations:

(1) Clinical lead poisoning has never been observed with a clinically negative blood lead (*i.e.*, below the critical level of 0.1–0.2 mg.);

therefore, a clinically negative result eliminates lead poisoning as a diagnostic consideration.

(2) Severe lead poisoning (convulsions, etc.) has never been observed with a clinically questionable blood lead (at the critical level of 0.1–0.2 mg.); therefore, a clinically questionable result makes severe lead poisoning an unlikely diagnostic consideration.

(3) A clinically positive blood lead (above the critical level of 0.1–0.2 mg.) has never been observed except in clinical lead poisoning or during convalescence from it; therefore, a clinically positive result indicates immediate or slightly remote lead poisoning.

SUPPLEMENTARY SPECTROGRAPHIC ANALYSIS OF ORGANS AND OTHER MATERIALS IN LEAD POISONING

Spectrographic analysis for lead was also used in the occasional examination of cerebrospinal fluid, urine, and organs. The spectrographic detection of lead in cerebrospinal fluid has been carried out by Kimura and Uchida (17) and by Rabinowitch, Dingwall, and Mackay (18). Spectrographic methods for lead in urine have been reported by Boyd and De (19) and, recently and with refinements, by Cholak (20). Gerlach and Gerlach (21), and quite recently Gaul and Staud (22), have reported the spectrographic determination of lead in tissues from cases of lead poisoning. The analysis of old autopsy material, particularly bones, may suggest a retrospective diagnosis of lead poisoning, although the degree of contamination from the preserving fluid must be borne in mind when dealing with such samples.

By means of a spectrographic method similar to that for the estimation of blood lead, a study was made of the distribution of lead in the fresh organs from the autopsies of two lead-poisoned children. The results, in general, were similar to those recorded by Aub, Fairhall, Minot, and Reznikoff (23). The lead was chiefly in the skeletal structures, with comparatively high concentrations also in the liver, pancreas, and brain. The spinal cord and the scalp (with hair) were examined in one case and appeared high in lead.

The spectrograph has also been used for the rapid and convenient analysis of paints, plasters, crayons, etc., which have been ingested by children. Such procedures have aided in tracing the probable sources of lead absorption.

DISCUSSION

Until recently the chemical aids to the diagnosis of plumbism consisted of very laborious methods for the determination of urinary lead. The procedures were obviously unsatisfactory for clinical purposes because several days were required for the completion of the analysis. New and simpler methods, based on the diphenylthiocarbazone ("dithizone") reagent, have been introduced by Bohnenkamp and Linneweh (24), Ross and Lucas (25), Sulkowitch (26), and Seelkopf and Taeger (27). The development of these procedures is making the determination of urinary lead more practical.

The comparative merits of blood and of urine analyses in lead poisoning are in dispute. From their extensive analyses of urine, Kehoe and coworkers (28) have concluded that urine analysis offers a much more accurate index of the lead in the body than does the analysis of blood, in which the lead concentration was considered to undergo comparatively great variation. Furthermore, these investigators did not find any correlation between the lead concentration of the excreta, blood, or other tissues, and the appearance of the symptoms of lead poisoning. Litzner and Weyrauch (9) have reached the opposite conclusion in that they found the results of urinary analysis to be variable and unreliable, found the determination of blood lead to be reliable, and disclosed a close relationship between the level of blood lead and the appearance of clinical symptoms. The results of our investigations have not been a comparison, but they have clearly demonstrated the reliability of blood-lead analysis for practical diagnostic purposes.

In regard to the choice of specimen source, it must be recognized that blood is a readily available fluid, while the collection of urine at times may prove inconvenient and cause delay, as in the case of dehydrated children. Blood, moreover, is of approximately uniform composition and a given sample may be considered representative, whereas a single specimen of urine cannot be regarded as a true sample. If a standard 24-hour sample of urine is awaited, a day is lost before the analysis may be started. Since the major part of the circulating lead appears to reside in the cell fraction (29), and it is probable that the ratio of ultrafilterable/non-ultrafilterable lead

may vary, the determination of urinary lead may be a questionable index of the lead level in the body.

The standard chemical methods for determining blood lead not only require several days time, but necessitate 50–100 c.c. samples. Withdrawal of such large amounts is uncertainly undesirable in adults and is obviously impractical for sick children. A promising improvement in this type of analysis may result from the quite recent report by Wilkins, Willoughby, Kraemer, and Smith (30), who have described a "dithizone" method which is more rapid and can be used with smaller samples.

The spectrographic method for the quantitative estimation of blood lead, which requires only 5 c.c. of blood and can be completed within 12 hours, has proved useful as a routine diagnostic aid. Electrometric measurement by a microphotometer may be substituted for the visual observation of line intensities in reading the plates, although this additional step has not been necessary for the clinical application of the method. Also, the time required could be reduced by the use of a less convenient wet digestion procedure, instead of simply ashing, for the destruction of the organic matter. For purposes of rapid diagnosis, however, a semi-quantitative modification has been described in the thirty-minute spectrographic micro-method for the detection of pathological lead in peripheral blood (31).

A chemical analysis is concerned only with the determination of lead. The spectrographic examination, on the other hand, not only determines pathological blood lead, but in the very same photograph presents in panorama most of the blood's inorganic constituents. Thus, the analysis occasionally may serve a dual purpose in diagnosis: first, by affirming or denying the presence of plumbism; and second, by revealing an abnormality of some other element. This has happened, for instance, in an unsuspected case of argyria with marked argyremia (32).

SUMMARY

(1) A method for the quantitative spectrographic estimation of blood lead, requiring 5 c.c. of blood and 12 hours for completion, has been described.

(2) Values for blood lead in health and in lead poisoning have been

established on the basis of analyses from 36 cases of clinical plumbism, and from the study of over 200 control samples.

(3) Non-pathological blood lead values (0.005–0.1 mg. per 100 c.c.) are considered clinically negative and eliminate lead poisoning as a diagnostic consideration.

(4) Pathological borderline blood lead values (at the critical level of 0.1–0.2 mg. per 100 c.c.) are considered clinically questionable, and indicate sub-clinical lead poisoning or mild clinical lead poisoning.

(5) Pathological blood lead values (0.2–1.0 mg. per 100 c.c.) are considered clinically positive and indicate clinical lead poisoning or convalescence therefrom.

(6) The majority of the cases of clinical plumbism showed blood-lead values in the range of 0.2–0.5 mg. per 100 c.c.

(7) During convalescence from lead poisoning and in the absence of additional exposure, clinical symptoms disappear before the blood lead falls to a non-pathological level. It appears that pathological blood-lead values may persist for 6–12 months after exposure, especially in children.

(8) Within the pathological range itself, there does not appear to be a close parallel between the level of blood lead and the severity of symptoms.

(9) The value of spectrographic and chemical methods in the diagnosis of lead poisoning has been discussed.

We wish to acknowledge the valuable suggestions of our former collaborator, the late Dr. Paul G. Shipley. We also wish to thank Professor E. V. McCollum and Professor E. A. Park for their suggestions, criticism, and constant encouragement.

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THE TREATMENT OF INTRACRANIAL HAEMORRHAGE RESULTING FROM CISTERNAL PUNCTURE

WALTER E. DANDY

From the Department of Neurological Surgery, The Johns Hopkins Hospital and University

Submitted for publication April 15, 1935

The following case is reported in order (1) to put on record one of the accidents that may occur from cisternal puncture; (2) to explain the reasons for its occurrence—reasons that make us wonder why it does not happen more frequently; (3) to describe the operative treatment by which the punctured artery is found and closed—without which life would surely be lost. The case is presented in some detail because it provides a fairly accurate experience from which the character, degree and duration of the resulting neurological disturbances may be estimated. It also affords an opportunity to evaluate the effects of a severe injury upon a syphilitic brain.

W. B., white, male, aged 47 (Surg. No. 55503). This patient had been treated in the Johns Hopkins and the Baltimore City Hospitals over a period of five years for general paresis. Owing to somewhat severe reactions from lumbar puncture, a cisternal puncture was substituted; the fluid was bloody. The patient soon complained of headache and became very restless; he was given morphine sulphate (gr. $\frac{1}{4}$) about half an hour after the puncture, at which time his pulse was 80 and the blood pressure 130/80. Twenty minutes later he was in coma; pulse 60; blood pressure 110/80. From a lumbar puncture at this time the fluid was very bloody and under pressure. The writer saw the patient about two and one-half hours after the cisternal puncture. He was then so deeply unconscious that he could not be roused by strong pressure over the supraorbital nerves; there was complete areflexia; the respirations were regular and quiet, 20 per minute; the pulse was 88. That there was an intracranial haemorrhage was, of course, recognized, but as another long operation had already been started, the attack upon the haemorrhage was seriously delayed. Three hours later when I again saw him his condition had changed greatly for the worse. Still profoundly unconscious, he was ghastly pale and somewhat cyanotic, clammy; the respirations were irregular, stertorous from mucus in the throat, and of the Cheyne-Stokes type; the pulse was still 88, but the quality varied markedly with the respirations, being very soft at the time when the breathing was depressed. The blood pressure

was now 150/76; temperature 99°F. Another hour was lost in persuading relatives to permit an operation. The operation was performed about six and one-half hours after the cisternal puncture (May 15, 1934). At that time it was obvious that he could not have lived much longer. Not even local anaesthesia was used; throughout the operation there was not the slightest movement. A bilateral cerebellar exposure was quickly made. On nicking the dura quite a quantity of dark blood escaped, doubtless from the subarachnoid space; the amount was so great that for the instant we thought it possible that a large vein or sinus might have been punctured. However, the blood soon ceased to flow and brain tissue then plugged the tiny dural opening. The needle was then passed into the lateral ventricle and clear fluid, with but a slight bloody tinge, escaped under some pressure. The absence of blood in the ventricular fluid was, of course, reassuring, for the great hazard of an intraventricular haemorrhage did not exist. Needless to add, the contrast between the ventricular and spinal fluids was extreme and must have been due to a closed foramen of Magendie.

The dura over the cerebellum was very blue, indicating a haemorrhage beneath. Upon opening the dura the blood was seen to fill the cisterna magna, and except for a thin film of blood over the cerebellar hemispheres was well confined to the subarachnoid space. The blood in the subdural space had doubtless escaped through the opening made by the needle used for the cisternal puncture. The blood, which was now in large part coagulated, was removed. There then began a brisk haemorrhage of arterial blood. Aspiration of the fresh blood with the continuous suction tube quickly brought into view the bleeding point in a small artery lying on the surface of the left cerebellar tonsil, the blood coming from its mesial surface to pass over the cerebellum. Alongside the bleeding point was a tiny black spot in the cerebellar tonsil; this represented the puncture wound of the needle which had just nipped the margin of the artery. The bleeding vessel was picked up with the forceps and coagulated with the electrocautery. No further bleeding occurred. The wound was closed without drainage.

Postoperative Course: Immediately after the haemorrhage had been removed the respirations became more regular and less labored and his color was improved. The first change in his profound deep coma appeared one hour later when he coughed owing to the presence of mucus in the throat. Two and a half hours later the arms and legs were moved and he would squeeze the hand when requested to do so. Four hours after the operation he was fully conscious and had full use of both arms and legs. Bilateral internal strabismus was then noted; the voice was hoarse; he was unable to swallow and had urinary retention. The knee kicks were slightly hyperactive and equal (despite his syphilis). Plantar stimulation provoked normal responses. He was disoriented for time and place, but not for persons; he had some hallucinations but they were not troublesome and soon passed away.

The return of functions was as follows: Swallowing began on the fifth day after operation; incontinence replaced retention on the same day, and several days later there was normal voluntary control. The strabismus steadily improved and

at the time of his discharge thirteen days after operation there was only slight weakness of the left external rectus. Although he was still disoriented at times, he remembered the cisternal puncture and the severe headache that followed. A slight dysarthria still persisted.

His wife reported a few months later that his mind had cleared and double vision had entirely disappeared. The patient, in her opinion, was in exactly the same condition, mentally and physically, as before the accident. At the time this is written, April 1, 1935, he is alive and in his preoperative normal condition.

Although the point of the needle went slightly to the left of the midline, the haemorrhage occurred only because of certain anatomical factors that were unusual but by no means uncommon (Fig. 1B). First, the tonsils of the cerebellum projected into the foramen magnum, thus greatly reducing the size of the cisterna magna, which otherwise could be easily reached with a needle and with scarcely any danger of encountering the brain stem or cerebellum. A fairly extensive experience in operating upon trigeminal neuralgia and Ménière's Disease, in both of which it is necessary to inspect and evacuate the cisterna magna, has taught that the cerebellar tonsils are not infrequently in the foramen magnum so that in these cases the cisterna magna is small and shallow, and we have often wondered why enthusiasts of cisternal punctures have not had more trouble from this cause. The reason for this variation can lie only in a congenital or early acquired difference in size of the posterior cranial fossa. When smaller than usual the space adjustment is made by the thrusting of the cerebellum downward through the foramen magnum. Indeed the tonsils of the cerebellum may be lodged so deeply in the spinal canal that, if an operative exposure of this region were made, one would be led to suspect a cerebellar tumor unless the variations in the normal were recognized. I have had at least two cases (both of trigeminal neuralgia) in which the anteroposterior dimensions of the posterior cranial fossa were reduced, at least, one-half, and in each the cisterna magna was obliterated as completely as if a large tumor had caused it (Fig. 2). Secondly, the haemorrhage occurred because a branch of the posterior inferior cerebellar artery chanced to be in the way; a similar vessel was not present on the other side. Given an absent cisterna magna or one reduced in size, variably placed branches of the posterior inferior cerebellar artery must be accepted as poten-

tial sources of injury from a needle even when it is almost perfectly directed.

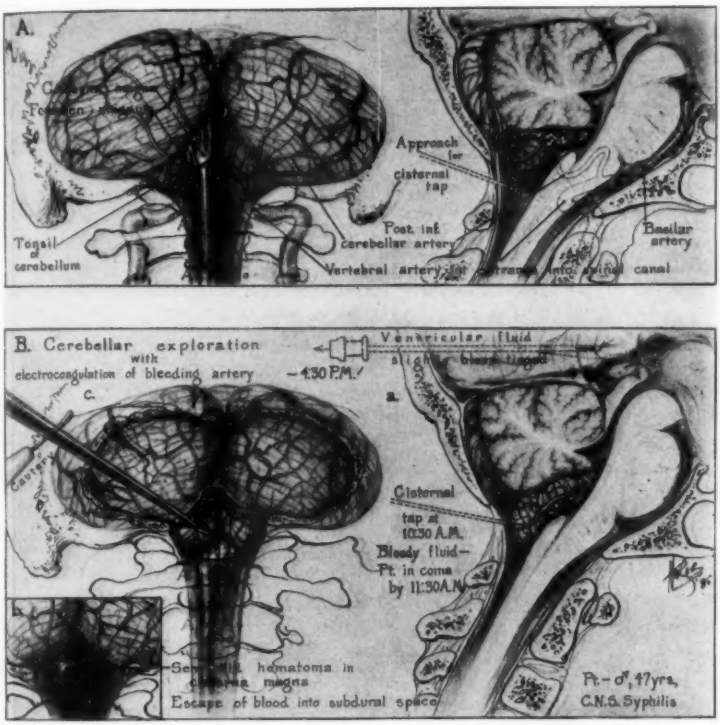

FIG. 1. (A) Showing normal cisterna magna. The cerebellar tonsils are well confined to the cranial chamber. (B) With limitations in the size of the posterior cranial fossa the cerebellar tonsils are forced into the spinal canal, thus obliterating the cisterna magna in varying degree. The branch of the posterior inferior cerebellar artery was punctured at the point indicated in the drawing. The bleeding vessel was thrombosed with the cautery.

While considering the dangers of cisternal punctures it is perhaps appropriate to mention a fatality that occurred in my own experience. To diagnose and localize a suspected spinal cord tumor, a cisternal puncture was attempted as a means of introducing lipoidal. Im-

mediately on passing the dura the needle entered the medulla, the entire body became rigid and death followed in a few minutes. Necropsy showed a tumor beneath the atlas; this mass had pushed the

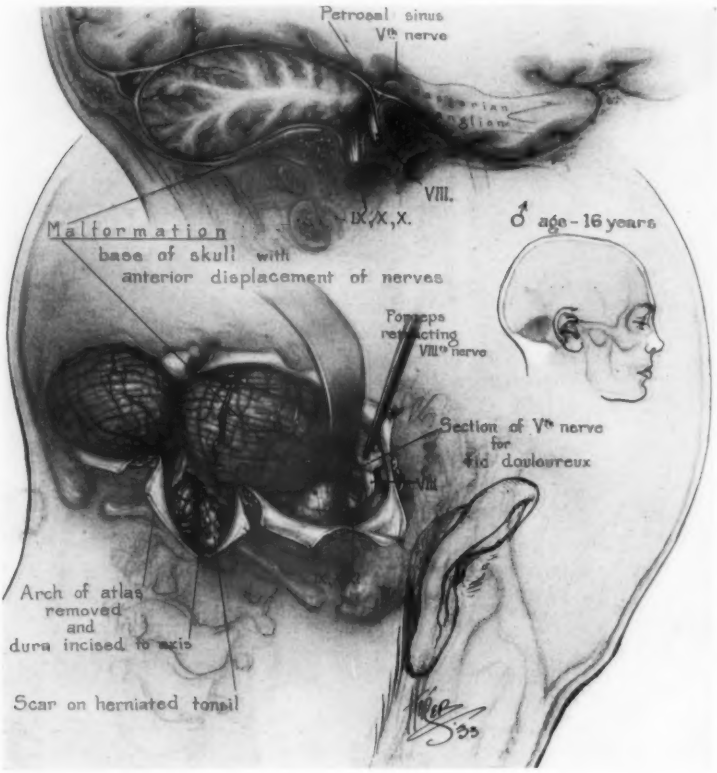

FIG. 2. An extreme congenital reduction in the size of the posterior cranial fossa with the cerebellar tonsils thrust far into the spinal canal. In such variations, which must be regarded as normal, cisternal puncture would be impossible and the attempt would be attended with a serious hazard.

medulla tightly against the dura and had, of course, obliterated the part of the cisterna magna lying between these structures.

The above statements are not to be regarded as indicating an

entire disapproval of cisternal puncture. Indeed there are times when it is indispensable—for instance, for the localization of spinal cord tumors with lipoidal. But one must accept a certain, though very small, risk in its use. Unquestionably a cisternal puncture is far less painful in performance and causes far less subsequent distress, including headache, than does a lumbar puncture. But the latter, at least, carries no risk to life or function (except in the presence of tumors of the brain and spinal cord, in both of which there is very serious danger), and should, therefore, be used routinely unless there is some definite contraindication. Doubtless when numerous punctures are required in treatment, the pain and discomfort resulting from lumbar punctures are adequate reasons for shifting to cisternal punctures, but the latter should certainly never be used when an intracranial tumor causing pressure is suspected (and this also holds good for a lumbar puncture) because, in the readjustment of the intracranial contents to the space occupied by the tumor, the cisterna magna is usually reduced and is frequently—*always* in the presence of large cerebellar tumors—entirely obliterated, so that the cerebellum then lies in direct contact with the dura at the point where the puncture will be made. With cisternal punctures the degree of risk will vary with the skill and experience of the operator, but even in the best hands accidents can not be avoided when anatomical variations place an artery in the path of the needle.

Returning to the case reported above, it is worthy of comment that the blood did not pass into the ventricular system; had it done so, recovery would scarcely have been possible. The same anatomical variations that obliterated, or at least greatly reduced, the cisterna magna, also necessarily closed the foramen of Magendie and prevented blood from passing into the fourth ventricle; and retrograde passage of blood through the foramina of Luschka is practically impossible because of the long passage through the narrow lateral recesses. From experiences with spinal air injections the inconstancy of the foramen of Magendie, at least as a wide open foramen, is known. Not infrequently air so injected will not reach the ventricular system because the foramen of Magendie is actually or potentially (as in the above case) closed, nor can the air reach the ventricular system through the foramina of Luschka, although it can pass freely in the

reverse direction through these channels, *i.e.*, from the ventricles to the cisterna lateralis. So much, therefore, is dependent upon the normal variations in the structural development of this region.

The diagnosis of an intracranial haemorrhage following a cisternal puncture is unmistakable. Drowsiness with severe headache, then coma, can scarcely be due to any other cause. Lumbar puncture is quite unnecessary for diagnosis and offers nothing in treatment, probably not even temporarily. Indeed it may act harmfully by again releasing the bleeding vessel which may have been closed by the clot that has packed over its mouth. A second lumbar puncture was done in this case in an effort to tide the patient over until operation could be performed, but it produced no appreciable change in his condition.

Although the direct attack upon a bleeding vessel is by far the most logical and satisfactory method of dealing with any intracranial haemorrhage, the occasions are rare indeed when it is possible. Not only is the intracranial pressure in large part relieved at once by evacuation of most of the haematoma, but the source of future bleeding is stopped. With the single exception of extradural haemorrhages resulting from a tear in the middle meningeal artery, the treatment of cerebral injuries, including haemorrhages, is indirect, *i.e.*, by control of the volume of intracranial contents through a decompression, because there is no way of determining the location of a bleeding vessel. Then, too, oedema and multiple small haemorrhages from lacerated brain tissue become a large but indetermined component in the total volume of increased intracranial pressure resulting from trauma—elements that do not enter with the *pure* arterial tears of the posterior inferior cerebellar and the middle meningeal arteries.

The profound and prolonged effect upon the brain of a sudden haemorrhage of six and one-half hours' duration is perhaps not unexpected, but the eventual complete disappearance of all the resultant signs and symptoms—including those of mentality—when general paresis pre-existed, is worthy of note, especially when undue emphasis is so frequently placed upon the relationship of trivial trauma as a precipitant of permanent sequelae from dormant cerebral syphilis.

Doubtless the time required for the subsidence of the neurological stigmata is in proportion to the rapidity of the haemorrhage and to

the time that has elapsed between the puncture and the operative relief. The earlier the operation the less severe and prolonged would be the after effects. There can scarcely be reason to doubt that not only would the danger to life have been far less but the convalescent period would have been greatly shortened, and the distressing after effects largely eliminated or at least greatly reduced, had the operation been possible as soon as the intracranial haemorrhage was recognized.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

The Patient and The Weather. By WILLIAM F. PETERSEN. [With the Assistance of Margaret E. Milliken.] 1934. Vol. II. 530 pp. $6.50. Vol. III. 1934. 375 pp. $6.50. (*Edwards Bros., Inc., Ann Arbor, Michigan.*)

In the second volume observations are presented to prove that functional disorders are intimately bound up with meteorological events.

The first part of the third volume offers a statistical survey, the second part, an analysis of patients suffering from various psychoses, the third, a study of neurological disturbances. Much stress is laid upon transient somatic findings coinciding with meteorological changes. The clinical material offered is interesting, but can hardly be called conclusive.

O. D.

French Medicine. By M. LAIGNEL-LAVASTINE and M. RAYMOND MOLINERY. [*Clio Medica*]. Translated by E. B. Krumbhaar. With 14 illust. 187 pp. (*Paul B. Hoeber, Inc., New York, 1934.*)

Translated by Dr. Krumbhaar, Editor of the Clio Series, this book begins with magical therapy and the Gallo-Roman Period. Chapter III discusses the real beginnings of French medicine, with an important section on the hospitals of the day, notably the Hôtel Dieu of Paris. Chapter V, which is extremely interesting, tells of medical teachings in France a thousand years ago, the various medical faculties, their powers and important functions. Chapter VI, on the Renaissance, is adequate, although comparatively little space is given to the influence of Paracelsus. Chapter IX, on the 19th Century, is naturally so over-loaded with material that it seems a mere succession of names and discoveries. More interesting is the chapter on Military, Naval and Colonial Medicine. Of course, French medicine no longer holds the important place that it held in the 18th and early 19th centuries, when all intellectual Europe spoke French.

There is an adequate index and the book is illustrated by 14 portraits.

J. R. O.

Dynamics of Population. By FRANK LORIMER and FREDERICK OSBORN. 461 pp. $4.00. (*Macmillan Co., New York, 1934.*)

This work presents a scientific, detached study in four parts of many of the difficult problems involved in the growth and character of the population in the United States. In Part I, the authors describe the population trends of American groups. Part II deals with the measurable characteristics of such groups. In

Part III, the influence of differential reproduction on the characteristics of the American people is discussed, and in Part IV the causes and control of population trends are considered.

The authors have no *a priori* views to advance but draw their conclusions from a thorough review of all the available reliable data, and are to be commended for having achieved a praiseworthy, scholarly representation of a subject of far-reaching importance to the future of our country.

J. H. M. K., Jr.

Conception Period of Women. By Dr. Kyusaku Ogino. [Translated by Dr. Yonez Miyagawa.] 1934. 94 pp. (*Medical Arts Publishing Co., Harrisburg, Pa.*)

This earnest booklet summarizes the literature concerning the fertile and infertile phases of the women's menstrual cycle. From a review of the literature and from human observations made by the author the following law is promulgated. "The human conception period is the 8 days from the 12th to the 19th day before the subsequent menstruation, notwithstanding the length of the menstrual cycles." The author's thesis, which is probably in the main correct, is greatly weakened by an unparalleled dogmatism.

A. F. G.

Methods of Treatment. By Logan Clendening. 1935. 5th edition. 879 pp. $10.00. (*C. V. Mosby Co., St. Louis, Mo.*)

This is the fifth edition of a well and interestingly written book covering procedures, which, in the opinion of the author, are most fundamentally sound in modern therapeutics. The revision has been thorough, all sections having been brought to recent date.

C. H. B.

Papers Relating to the Pituitary Body, Hypothalamus & Parasympathetic Nervous System. By Harvey Cushing. 1933. 234 pp. Illust. (*Chas. C. Thomas, Springfield, Ill. & Baltimore.*)

This monograph brings together and elaborates in further detail Cushing's four previously published lectures on what he terms "the long neglected interpeduncular region of the brain." It is an admirable summary, not only of his own original contributions, but also for pertinent literature up to the date of publication.

M. B. W.

BOOKS RECEIVED

The New Dentistry. A Phase of Preventive Medicine. By Leroy Matthew Simpson Miner. 1933. 219 pp. $2.00. Harvard Univ. Press, Cambridge, Mass. [Six Lowell Lectures.]

Mayou's Diseases of the Eye. By FREDERIC RIDLEY & ARNOLD SORSBY. 1933. 4th ed. 249 pp. $2.25. Oxford Univ. Press, New York.

Mary Imogene Bassett Hospital. Clinical Miscellany. 1934. Vol. 1. 206 pp. $3.00. (*Charles C. Thomas, Springfield, Ill. and Baltimore, Md.*)

A new periodical containing twenty-two clinical cases by different hands.

F. H. G.

JAUNDICE PRODUCED BY A DIVERTICULUM OF THE DUODENUM

WILLIAM M. NICHOLSON

From the Department of Pathology, Johns Hopkins University, and Medical School

Submitted for publication April 17, 1935

Diverticulum of the duodenum which obstructs the bile duct is a rare cause of jaundice. The following is a report of a case in which this condition was discovered in an elderly white man.

CASE REPORT

J.B. Unit No. 45,359. A white, retired fireman, aged 66, entered the Medical Clinic of the Johns Hopkins Hospital, on September 14, 1932, complaining of jaundice and epigastric discomfort. His illness dated approximately from eight months prior to his admission to the hospital when he began to notice epigastric discomfort and eructations soon after eating. Jaundice appeared four and a half months before admission. At about the same time the patient discovered that fatty foods were distasteful, so much so that he frequently vomited following the ingestion of a meal in which there was only a moderate amount of fat. The vomitus contained only undigested food. At no time did it contain blood nor was it bile-stained. After the onset of jaundice he became conscious of a dull, aching pain in the right upper quadrant, which did not radiate and usually came on about one hour after eating. About one month later he noticed that his abdomen was swollen. During this entire period his stools were very light in color; his urine dark brown. Another interesting feature was that the jaundice would be less marked on some days than on others. During the eight months previous to admission he lost fifty pounds in weight. Aside from the symptoms referable to the gastro-intestinal tract, he suffered from dyspnea, orthopnea and edema.

Examination revealed a large-framed, emaciated, white man of 66 years, in moderate respiratory distress. There was deep jaundice of the skin and mucous membranes. No spider angiomata were seen. The blood pressure was 144/80. The heart was slightly enlarged, the aorta dilated, and a systolic murmur was audible over the whole precordium. The abdomen showed the typical signs of ascites. A firm, irregular mass was palpable in the right upper quadrant, and, following abdominal paracentesis, another rounded mass was made out immediately below and apparently attached to the irregular mass. These masses were felt to descend upon deep inspiration and were thought to be the liver and the gall-

bladder. The spleen was not palpable. With the exception of the moderate edema of the ankles, the remainder of the examination revealed nothing of interest.

The results of the laboratory examinations were as follows: R.B.C. 3,100,000; Hb. 76%; W.B.C. 8,000. The differential count was normal. The Wassermann test was positive. The stools were consistently gray and acholic, but were free from microscopic and occult blood, except during the terminal stage of his illness when he began to vomit. The urine showed large amounts of bile salts. The bromsulphthalein liver function test showed 30% retention of the dye at the end of thirty minutes. The Van den Bergh reactions were:

September 8, direct reaction	11.4 mgm. %
September 15, prompt biphasic reaction	5.7 mgm. %
September 22, prompt biphasic reaction	5.7 mgm. %
September 28, prompt biphasic reaction	5.4 mgm. %

The x-ray report of the gastro-intestinal series is as follows; "No evidence of lesions in the stomach or duodenum. Duodenal cap large; no deformity. Second and third portions of the duodenum do not present findings suggestive of carcinoma of the head of the pancreas, but there is a large diverticulum at the junction of the second and third portion of the medial aspect. This is still filled at the end of five hours."

The temperature and pulse remained at normal levels during his stay on the ward.

On September 24, the patient began to vomit blood and in spite of two blood transfusions, he became weaker, and died on September 30.

Clinical Diagnosis: Syphilis, Syphilis of Aorta, Carcinoma of Pancreas (?). Syphilis of Liver (?).

Autopsy report. Path. No. 12808. Autopsy performed about one hour after death. The body was that of a large white man in only a fair state of nutrition. There was some edema of the lower extremities. Deep jaundice was noted over the entire body. The abdominal cavity contained approximately 4.5 liters of dark yellow fluid. The liver edge was 3 cm. below the costal margin, beneath which the gall-bladder extended for a distance of 7 cm. The liver was much enlarged, but no accurate weight was obtained, since the gall-bladder, duodenum and pancreas were left attached. The liver had been converted into a nodular mass, and the individual nodules were from 2–6 mm. in diameter. The color ranged from a deep brown to various shades of green. Between the nodules the tissue was gray and translucent. The gall-bladder was distended with 300 c.c. of dark green bile; the walls were thin and transparent. The bile ducts were dilated and bile-stained. The common duct was 12 mm. in diameter down to a point about 1 cm. from the Ampulla of Vater. From this point onward the duct was markedly constricted. Upon careful examination no stones were found in the bile duct, the cystic duct, or the gall-bladder. Partly surrounding the common duct, near its entrance into the duodenum, was a crescent-shaped diverticulum of the duodenum, the end of which was firmly attached to the common duct and the

outer wall of the duodenum by bands of fibrous adhesions. The diverticulum was about 3 cm. long and about 1.5 cm. in diameter. The opening of the diver-

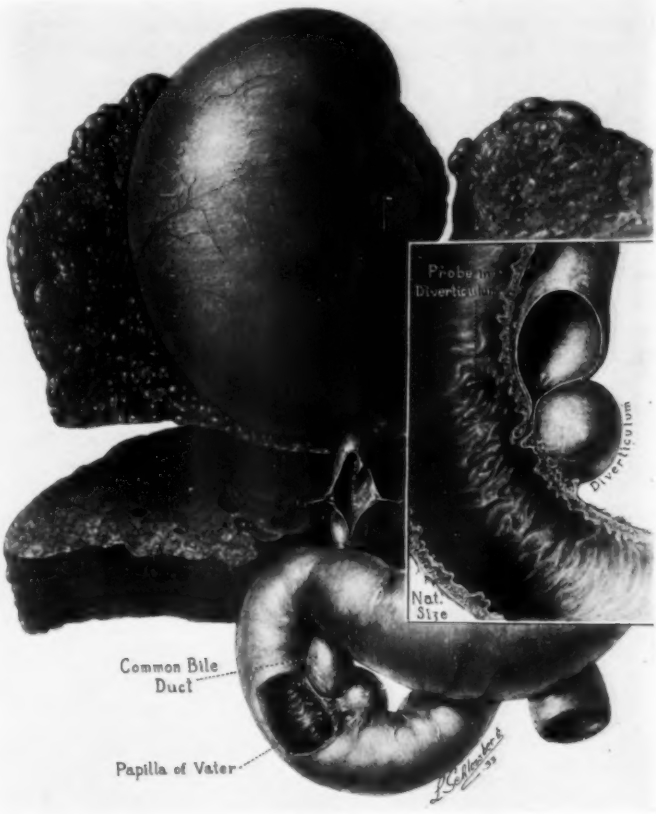

FIG. 1

Figure 1 shows the enormously enlarged gall-bladder, and the dilated cystic and common ducts. The course of the common duct, and its entrance into the duodenum where it is surrounded on the one side by the diverticulum and on the other by the duodenum, are shown. The insert shows the relative size of the duct and the diverticulum at the point of constriction. The pancreatic duct, which is not shown, was situated at the distal end of the diverticulum and was therefore not occluded.

ticulum into the duodenum was about 7 mm. distant from, and was only slightly larger than, the opening of the Ampulla. When a longitudinal section was made through the diverticulum, it was discovered that it formed an acute angle on itself, this angle was about 5 mm. from the opening into the duodenum. The fold in the wall of the diverticulum, so formed by the angle, projected inward in such a manner as to act like a valve.

Removal of material from the diverticulum for microscopic study was postponed until the drawing was completed. Unfortunately, the specimen was not well preserved and became unfit for microscopic study. However, it might be said that the diverticula occurring in the region of the Ampulla of Vater or the "perivaterian" diverticula, are of the acquired type, and do not contain muscle fibers in the fundus of the diverticulum, but only at the mouth (1, 2).

Microscopic examination of the liver showed marked periportal scarring, with bands of connective tissue running throughout the small nodules of liver cells. The liver cells were partly filled with bile pigment, and many contained iron as shown by the stain. The bile capillaries were dilated. The pancreatic ducts were not distended and no areas of necrosis were seen in the pancreas.

The aorta showed the typical findings of syphilis, both in the gross and in the microscopic examinations.

Anatomical diagnosis: Diverticulum of duodenum; distention of common bile duct and gall-bladder; cirrhosis of liver (biliary?); jaundice; oesophageal varices; hæmorrhage into stomach; ascites; enlarged mesenteric lymph nodes near neck of the gall-bladder. Syphilis of aorta; fusiform dilatation of arch of aorta; cardiac hypertrophy; scars in myocardium. Arteriosclerosis. Emphysema. Hyperplasia of bone marrow. Prostatic hypertrophy.

DISCUSSION

Diverticula of the duodenum are not rare; they occur most frequently in the region of the Ampulla of Vater. For a discussion of the subject one is referred to Hahn's (2) extensive review, in 1930.

The following case reports have been found in which a diverticulum of the duodenum was associated with jaundice or with cirrhosis of the liver, confirmed at post-mortem examination or at operation. Those cases in which a stone was found in the common duct have not been included. Roth (3), in 1872, described the autopsy findings in a patient with a duodenal diverticulum, cirrhosis of the liver, and atrophy of the pancreas. Buschi (4), in 1911, found slight cirrhosis in a case reported by him. Wilkie (5) in 1913, found a large gall-bladder with dilated ducts. In this case a small stone was found in the gall-bladder but there were none found in the ducts. Ohnell (6), in 1923, recorded a case in which the diverticulum was diagnosed by means of the x-ray. This was later confirmed at post-mortem examination,

and cirrhosis of the liver was also present. Stepp and Gutzeit (7), in 1930, reported two cases in which the diagnosis was made clinically by means of the x-ray; at operation no stones were found in either case. One of the patients died and the diagnosis at autopsy was "cirrhosis of the liver." In 1932, Kohler (8) gave the case histories of 8 patients with diverticula of the duodenum. In one case a stone was found in the common duct. Jaundice was present in five other patients, two of whom died. The results of the post-mortem examinations were given in these two cases. Although in each case a diverticulum was found to be closely attached to the common duct, there was no dilatation of the ducts or cirrhosis of the liver. Gibbons (9), in 1933, reported 20 cases of diverticula of the duodenum, but only one of the patients had jaundice. Friedenwald and Feldman (10), in the same year, reported 34 cases of diverticulum and in three instances jaundice was present. No mention is made of the pathological studies.

The obstruction to the bile passages was produced, in all probability, in the following way. Repeated filling of the diverticulum over a period of years by intestinal contents appears to have resulted in thinning out of its wall and in seepage of material through the thinned wall, since there were dense adhesions between the diverticulum and the duodenum. After the formation of the adhesions the diverticulum became firmly attached to the duodenum in such a way as to form an acute angle. The fold so formed by the angle projected into the lumen of the diverticulum and acted like a valve, which held back some of the intestinal contents after it had entered the diverticulum. Consequently, there was constantly some dilatation of the latter. When the diverticulum dilated there was compression of the common duct between the diverticulum and the duodenum. It is interesting that the jaundice was intermittent in character. This may have been the result of variations in the degree of distension of the diverticulum. It is true that the jaundice did not entirely disappear after it had once appeared, but clinically, and by the Van den Bergh reaction, it was observed to fluctuate.

From the microscopic appearance of the liver, one cannot be sure that the cirrhosis was of the biliary type. It is our opinion, however, that the obstruction had extended over a long period of time, and that the cirrhosis had progressed to such an extent that it was impossible

to state definitely whether a biliary cirrhosis had preceded the present picture. It is well known that long-standing obstruction of the bile passages can produce a cirrhosis of the liver exactly like that seen in diffuse nodular cirrhosis.

The only means by which one can make the diagnosis of diverticula of the duodenum is with the aid of the x-ray examinations, and from the many case reports in the current literature this diagnosis is being made with increasing frequency. The symptoms in many instances have been relieved by the surgical removal of the diverticulum. It is suggested that surgical exploration should be made in such patients when obscure jaundice of the regurgitative type is present.

SUMMARY

1. A case of duodenal diverticulum is reported in which intermittent jaundice was caused by compression of the common bile duct by the anomaly.
2. Attention is called to similar cases in the literature.
3. X-ray studies of the gastro-intestinal tract are urged in all cases in which there is an obscure jaundice.
4. Surgical removal of the diverticulum offers a feasible plan of treatment.

I wish to express my thanks to Mr. Max Bröedel of the Department of Medical Art and especially to Mr. L. Schlossberg who made the drawing.

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THE PLASMA-CELL PARTITION OF BLOOD LEAD IN CLINICAL LEAD POISONING

HAROLD BLUMBERG, Sc.D.[1] AND T. F. McNAIR SCOTT, M.R.C.P. Lond., M.R.C.S. Eng.

From the Biochemical Laboratory of the Johns Hopkins University School of Hygiene and Public Health, and from the Harriet Lane Home of the Johns Hopkins Hospital, and the Department of Pediatrics of the Johns Hopkins University School of Medicine

Submitted for publication April 17, 1935

During the course of early investigations on the spectrographic detection and estimation of blood lead in lead poisoning (1, 2), an attempt was made to concentrate the lead by separating the serum from the clot. In view of the work reported by Aub, Fairhall, Minot, and Reznikoff (3), who found that the greater part of the blood lead in experimental lead poisoning was held by the plasma, and but little by the cells, and in consideration of the general similarity between the chemistry of calcium and that of lead, it was expected that the serum would show an increased lead content. The analysis, however, revealed that by far the greater part of the lead was in the clot fraction, and only a little in the serum. In consequence of this observation, a series of studies has been made during the past two years to determine the plasma-cell partition of blood lead during its transport in clinical lead poisoning.

PARTITION ANALYSES

The material for this investigation consisted of blood samples from 18 cases of clinical lead poisoning, most of which were in children. Analyses were made by the spectrographic method as previously described (4), except that the sample was increased from 5 c.c. to about 8 c.c. to provide for sufficient analytical material after separation.

The first distribution studies were made upon serum-clot separations, and the results confirmed the original observation that the greater part of the lead, usually about 90%, appeared in the clot.

[1] Fellow in Medicine of the National Research Council, 1934–35.

In order to eliminate the possibility of partition changes during the clotting process, the majority of the analyses were done on plasma-cell fractions which were secured by immediate centrifugation and careful separation of oxalated, citrated, and heparinized samples. Again the greater portion of the lead was shown to be associated with the red cells (*cf.* fig. 1).

When distribution analyses were carried out upon control bloods which contained faint non-pathological traces of lead, essentially the same results were obtained. Because of the extremely small amounts of lead present, the results could not be striking. The cells (or clot), however, always appeared to contain as much lead as the plasma (or serum) and usually showed much more.

The use of the anticoagulants, oxalate and citrate, might be viewed with suspicion because of the possibility of their precipitating the lead onto the cells. That such an action is unlikely is shown by the fact that the partition of the blood calcium, which is the chief reactant, suffers no great alteration, particularly with the citrate. Furthermore, the use of heparin circumvents this objection, although it introduces a slight trace of lead as a contaminant. A comparison of the lead content of the heparin with the lead content of the cells easily demonstrated that the impurity could not be great enough to affect the distribution.

In the study of the non-pathological lead traces in control bloods, it was possible to verify the plasma-cell partition in the absence of an anticoagulant by recourse to hemophilic blood.[2] This analysis again revealed that the greater part of the lead (approximately 90%) was in the cell fraction (*cf.* fig. 1). A similar study on pathological amounts of blood lead was not made, however, because such an extremely unlikely case—that of a hemophilic with lead poisoning—was not available.

The major portion of the lead having thus been associated with the red cells or clot, the following experiment was undertaken to determine whether or not the lead was merely entrained by the cells in a loose combination. Immediately after separation from the plasma, the cell fraction was shaken with lead-free 0.9 N. NaCl

[2] We are greatly indebted to Mr. Jesse Yaffa for generously supplying a sample of hemophilic blood.

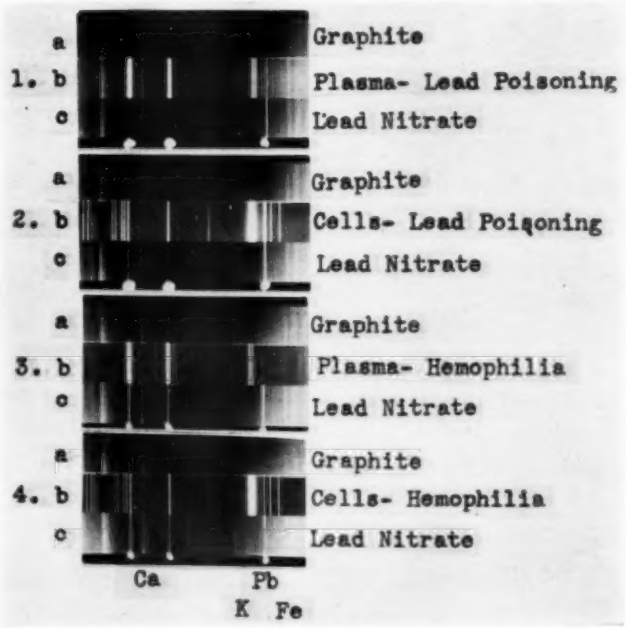

FIG. 1. The dot on the right marks the position of the lead *raie ultime*, or most persistent line, at 4057.83 Angstrom Units. The two dots on the left mark the positions of the calcium lines at 3933.67 A.U. and 3968.48 A.U. A little to the right of the lead line are the two iron lines at 4063.76 A.U. and 4071.90 A.U. Slightly to the left of the lead line, and quite close to each other, are the potassium lines at 4044.16 A.U. and 4047.22 A.U.

1. In the first spectrogram, *a* is the control spectrum of the empty graphite electrodes; *b* is the heparinized plasma ash from a case of lead poisoning, and *c* is a drop of a lead nitrate solution to supply reference lines.

2. *b*, Heparinized cell ash from the same blood sample. Notice that the lead line is much heavier in the cells than in the plasma, while the reverse is true for calcium. The blood lead had a content of 0.5 mg. per 100 c.c., approximately 90% of which was in the cell fraction.

3. *b*, Plasma ash from hemophilic control.

4. *b*, Cell ash from hemophilic control, from same blood sample. Notice that the lead line is clearly visible in the cells, but not in the plasma. The blood had a lead content of 0.01 mg. per 100 c.c., approximately 90% of which was in the cell fraction.

solution, centrifuged, and again separated at once. This procedure was repeated, and analyses were made on the four portions: plasma, first saline washings (containing the residual plasma), second saline washings, and washed cells (containing residual saline). The results showed that very little of the lead had been removed from the cells by two saline washings, thus indicating that the metal was in rather firm combination with the red cells.

DISCUSSION

Experimental studies on the plasma-cell partition of blood lead have been recorded in the literature. Behrens and Pachur (5) injected animals with $PbCl_2$ containing a small amount of a radioactive isotope. When the blood was withdrawn from the animal a short time later and its lead content determined by using the radioactive lead as an index, it was found that by far the greater portion of the metal was contained in the cells. Behrens (6) assumes that the radioactive lead isotope behaves like ordinary lead in the animal body. Although this assumption is probably correct, the recent observations on the toxicity or modified action of "heavy water" (deuterium oxide), reviewed by Barnes and Jahn (7), have demonstrated the possibility of striking differences between the physiological chemistry of a common element and that of its isotope.

Bischoff and coworkers (8) injected animals with lead compounds and analyzed the blood half an hour later. In the case of colloidal lead triphosphate injections, 10 times as much lead was carried by the cells as by the serum, while the reverse was true for highly ionized compounds. Thus it appears that the distribution in experimental lead poisoning may vary under different conditions of injection.

Such experiments, however, do not show the conditions present in the ordinary type of clinical lead poisoning. As previously mentioned, Aub and coworkers (3) found that the greater part (practically 80%) of the lead was in the plasma in their lead poisoned experimental animals. Approximately the opposite distribution was observed in our 18 cases of clinical lead poisoning. The difference in results might be due to the severity of the experimental lead poisoning, in which the absorption of a very large amount of lead, greater than

that encountered in the usual type of human lead poisoning, might alter the conditions of distribution. In a few children the plasma lead appeared to increase somewhat during convalescence, and suggested a possible correlation with Vitamin D administration; but in no case did the plasma lead become greater than the cell lead. During the latter part of our investigation some experimental evidence consistent with our results was reported by Weyrauch, Necke, and Müller (9), who found, in an experimentally lead poisoned goat, that over 90% of the lead was in the clot. The low concentration of lead in the plasma fraction is in harmony with the fact that comparatively small amounts of lead are excreted in the urine during plumbism.

The striking difference between the plasma-cell partition of calcium and that of lead well illustrates the need of caution in extending the chemical analogy between the two elements to their physiological behavior.

In view of the concentration of lead in the cell (or clot) fraction, and the possibility of changes in the distribution ratio, it seems probable that the analysis of plasma (or serum) alone might not provide a safe index of the extent to which the blood is "leaded." For these reasons it has been seen fit to employ whole blood, rather than serum or plasma, in our work on the quantitative spectrographic estimation of blood lead in the diagnosis of lead poisoning (2) (4).

SUMMARY

(1) The plasma-cell partition of blood lead was studied in 18 cases of clinical lead poisoning by spectrographic analyses of the separated fractions from oxalated, citrated, and heparinized samples, as well as by analyses of serum and clot.

(2) The results showed that by far the greater part (usually about 90%) of the lead appeared to be carried by the cells or clot.

(3) Analyses of control bloods, including a hemophilic sample, indicated that the cell fraction contained at least half, and usually much more, of the trace of non-pathological blood lead.

(4) Washing the cells twice with isotonic saline failed to remove appreciable quantities of the lead, thus demonstrating that the lead and the red cells were in comparatively firm combination.

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RESEARCHES ON TETANUS

III. Further Experiments to Prove That Tetanus Toxin Is Not Carried in Peripheral Nerves to the Central Nervous System[1]

JOHN J. ABEL, B. HAMPIL AND AUGUST F. JONAS, JR.

From the Laboratory for Endocrine Research, the Department of Bacteriology of the School of Hygiene and Public Health, and the Department of Surgery of the Johns Hopkins University

Submitted for publication April 30, 1935

INTRODUCTION

In two earlier communications (1) (2) of our series on tetanus we presented many facts in support of our belief that the toxin of *clostridium tetani* is not transported in peripheral nerves to the central nervous system, but that, in common with other water-soluble and locally non-injurious substances, it can be distributed throughout the body by the blood stream only. It was pointed out that it is thought that when suitable quantities of the toxin are injected into the sciatic nerve of a cat or rabbit, as has been done innumerable times in the past fifty years, local contractures in many or all of the muscles of the corresponding extremity always make their appearance in due course. It is believed by the adherents of the central theory that this local tetanus appears because the toxin is carried to corresponding motor cells of the cord by way of the injected sciatic nerve. We hope to show by experiments on an animal with larger sciatic nerves, such as the dog, that it is possible to inject considerable quantities of toxic filtrates from cultures of *clostridium tetani* directly into the sciatic nerve of this animal without causing the slightest symptoms of local tetanus; and also that the neglect to observe certain precautions will lead to the escape of toxin from the injected part of the nerve and the inevitable appearance of local tetanus in the muscles in its immediate

[1] A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of investigations on tetanus.

vicinity. In a word, local tetanus of the muscles of an extremity whose sciatic or other nerves have been intraneurally injected with the toxin, appears, not as a consequence of a centripetal neural carriage of the toxin, but only because the toxin that has escaped from the part of the nerve into which it had been injected has entered these muscles. It is next to impossible to perform intraneural injections in the case of the slender nerves of a cat or rabbit without inducing localized leakage from the injected part of the nerve. As the sciatic is wrapped about with muscles, as it were, this escaping fraction of the toxin is, proportionally, just as effective in inducing local tetanus as if it had been injected directly into the muscles or into the spaces between them. As was shown in papers I and II, that fraction of the toxin that does not escape from the site of the intraneural injection is carried to the blood via the neural lymphatics and not to the spinal cord, as was thought to be the case.

As far as we can learn, only one of the early experimenters, Zupnik (3), succeeded in making intraneural injections of tetanus toxin that were not followed at all by local tetanus; in place of this there appeared only signs of descending tetanus. It will be recalled that this student of tetanus was the first to show that local tetanus does not appear in experiments with laboratory animals when the toxin is injected into areas from which it cannot readily come in contact with voluntary muscles. An area of this kind is the muscle-free space between the tibia and the tendo Achillis. When injected into this space the toxin is slowly carried away into the blood stream by the many lymphatic networks of the connective-tissue structures lining the space. We have referred in our first paper to the great significance of this observation.

Forty years ago it occurred to Zupnik, in experiments on dogs, to use the part of the posterior tibial nerve lying in this muscle-free space for his intraneural injections of toxin. It is evident that any fraction of the toxin that might escape from the injected nerve could not induce local tetanus, since injection of it into this muscle-free space is also not followed by local tetanus in the region of the hock, unless, as was well known to Zupnik, too large an amount of the toxic solution is here injected. The selected nerve, the posterior tibial, was exposed by an incision that began close to the *os tarsi fibulare* and was carried

up parallel and close to the tendo Achillis. We omit all details of the operation such as the employment of ligatures and other precautions. A few hundredths of a cubic centimeter of a powerful solution of the toxin were slowly injected peripheralwards into the nerve. Nevertheless, despite all precautions, the nerve was markedly distended at the point of injection and discolored by the dark solution employed. After having overcome certain difficulties that attended his first trials, such as stiffness at the hock, Zupnik reports that the only symptoms that followed his successful injections into the posterior tibial nerve were confined to contractures of the muscles of the face and head, of deglutition and of mastication. The trismus developed later than the dysphagia. Zupnik concluded from this experiment that tetanus toxin is not carried by way of peripheral nerves to the central nervous system, but in so far as it escaped from the injected nerve (and that it did so cannot be doubted), he believed that it was carried by the blood stream to the motor centers of the facial and other nerves that control the muscles of the face and head and whose contractures, as stated, were very pronounced. Zupnik's contemporaries paid no attention whatever to his experiment as here outlined. The reason for this may lie in the fact that, in the first place, many of his other experiments could not be successfully repeated and, in the second place, because very probably the experiments with the posterior tibial nerve were interpreted in the following manner: because Zupnik used ligatures to confine for a time the considerable volume of toxin injected into the peripheral part of the nerve, it may have been assumed that practically all of it escaped into the spaces that contained no muscles and no other motor nerve than the one that was injected. Lymphatics of these spaces could carry the toxin into the blood stream, and by this way it would reach and be absorbed by the terminals of the short motor nerves that innervate the facial and other muscles of the head, and, being transferred by these nerves to the corresponding motor centers, it would induce the observed contractures, in the usual manner, as postulated by the theory of the purely central action of the toxin. The validity of the criticism is weakened by the implied assumption that *all* of the toxin escaped from the injected part of the nerve. But whatever may be the reason why the description of this experiment passed into oblivion, it certainly de-

serves to be recalled to mind. We believe that our own method of demonstrating that toxin is not carried by peripheral nerves to the central nervous system is free from objections that were presumably offered by the upholders of the purely central theory against the evidential value of Zupnik's intraneural injections. We have selected the sciatic nerve for our injections at a point above its division into the tibial and peroneal nerves. Any escape of toxin at this point manifests itself in a few days by inducing local tetanus in muscles lying close to the point of injection, as none of the escaping toxin can reach a region that is not supplied by muscles and motor nerves.

The experiments here presented prove once for all, we believe, that when proper precautions are taken in working with a larger sciatic nerve, such as that of the dog,[2] the injected toxin neither escapes from the injected nerve in quantities that suffice to poison the muscles in its vicinity, nor is it carried by any component of this nerve to reacting cells of the spinal cord. We must conclude, as will be seen from a study of our protocols, that intraneurally injected toxin, *when its local escape has been avoided*, is conveyed in its entirety away from the nerves by their lymphatic trunks to the venous system. This being the case, we cannot expect any evidence of a central tetanic action from the quantities of toxin that have entered the blood stream by way of the neural lymphatics of the injected nerve, unless a fatal or nearly fatal dose has been injected. It has long been established that one can inject, intravenously, quantities of toxin that lie considerably below the minimal lethal dose without inducing symptoms of local tetanus such as always appear after subcutaneous or intramuscular injections of equivalent doses.

It will be shown that when we confine ourselves to injecting 1/50, and even larger fractions, of a lethal dose into a sciatic nerve, neither local tetanus nor any symptom of descending tetanus was evoked, although the animals were observed daily for many weeks thereafter (table III). In accordance with what is now known concerning

[2] Naturally, the much larger sciatic nerve of the horse would serve still better for intraneural injections of toxin; and it might even be possible to cannulate one of the lymphatic trunks of the nerve and collect lymph containing toxin. The outflow of lymph from the injected sciatic of the bound and anaesthetized horse might be hastened by faradic stimulation of the muscles of the leg.

the lymph flow of nerves, we must believe that the entire amount of the intraneurally injected toxin, in all experiments in which leakage from the nerve is avoided, is carried away via neural lymphatics into the blood stream. It can be shown that this quantity of toxin can be injected directly into a vein without eliciting any symptoms whatever, for the simple reason that it is absorbed and bound very largely by the non-reactive tissues of the body. But this 1/50 of a lethal dose, so entirely innocuous when injected into a sciatic nerve, nevertheless suffices to induce the most pronounced rigidity of all the muscles of two hind legs or even of all four extremities of a control animal, when injected intramuscularly into them at many separate points.

METHOD

The experiments here detailed were carried out on fairly large adult dogs of both sexes, varying in weight from 10.6 to 22.27 kg. It is evident that the much larger sciatic nerves of the dog serve better for intraneural injections than the more slender sciatics of the cat or rabbit. An analysis, as given in paper II of our series, of the work of our predecessors who made use of these smaller animals, shows that it is practically impossible to inject their sciatics without leakage from the injected part of the nerve.

After subcutaneous injection of 16 mg. of morphine sulphate, the dogs were anesthetized with ether and the lateral surface of the left thigh was shaved, washed with ether and sterilized with tincture of iodine followed by 70 per cent alcohol. Using a rigid aseptic technique a long incision was made longitudinally in the thigh, the muscular septum between the *M. biceps femoris* and the *M. vastus lateralis* was spread apart, and the sciatic nerve exposed. The nerve was then dissected free from the surrounding connective tissue and muscles, care being taken to injure no branches. It was next elevated from the surrounding muscles by passing several layers of moist gauze beneath it, and was then completely packed off from the muscles by more layers of gauze moistened in normal saline solution. With such a preparation of the field it is impossible for tetanus toxin to come in direct contact with any muscle during its injection into the nerve trunk.

The injection needle was introduced as nearly as possible into the

center of the nerve trunk and was then pushed 6 to 8 mm. along its main axis before injection of toxin was commenced. Injection required 10 to 20 seconds. Following withdrawal of the needle, the point of entrance was always carefully observed, to note any backward leakage of the toxin. Although such leakage was seldom actually observed, the nerve was, nevertheless, always repeatedly sponged with moist gauze pledgets for at least 10 minutes before the protective gauze packings were removed, except in the two experiments of Group II. The nerve was then replaced and the skin closed with interrupted silk sutures. In dogs S-13 and S-14 in Group III the nerves were left exposed for 30 minutes and gently sponged from time to time before the wound was closed.

The muscular stiffness of a limited number of muscles, as reported in the protocols of Groups I and II, is due, we believe, to the escape of a small amount of toxin, either by way of the hole made by the hypodermic needle or by seepage through the perineural membrane. Since it is not unlikely that escape of toxin from an injected nerve is enhanced by the massaging action of muscular activity[3] in walking and running about, the movements of ten of the injected animals were restricted for several days following the operation. This was effected by confining each of these animals to a small cage, while others of the injected group were allowed to run free in the paddock.

In injections of this sort the ordinary tuberculin syringe proves inadequate. Not only are these syringes too roughly graduated to permit the injection of *minute* quantities of toxin, but their closely fitted pistons offer so much resistance that we cannot always inject very gradually a minute amount of a solution, without a mishap.

The use of a graduated glass serological pipette has obviated both of these difficulties and proved entirely satisfactory. This pipette, 40 cm. in length, is graduated to 1/1000 of a cubic centimeter. A small hypodermic needle, gauge 23, is secured to its lower end with molded beeswax, while its upper end is closed by a short section of stoppered rubber tubing which acts as a bulb and controls the flow of fluid as it is gradually compressed.

[3] See page 125 of our first paper (*Science*, February 4, 1934) on the effect of work in hastening and intensifying the action of the toxin.

TABLE I

Group I. Showing results of the injection of doses of toxin ranging from 1/9 to 1/2* M.L.D. *into the left sciatic nerve of each of four dogs, in total volumes varying from 0.025 to 0.083 cc., with stiffness of muscles confined to those nearest the site of injection but with no central manifestations of tetanus*

DATE	DOG NUMBER	WEIGHT	TOTAL VOLUME INJECTED	TOXIN IN M.L.D.'S	RESULTS	CONTROL EXPERIMENTS ON THE SUSCEPTIBILITY OF THE ANIMALS TO TOXIN
1935		*kg.*	*cc.*			
Mar. 6	S-8	18.41	0.03	1/9	Stiffness of left hind extremity of each animal began on 6th day; entirely confined to posterior thigh muscles. Knee and hock joints easily flexed. Z-position† readily enforced. Tendon reflexes normal. Period of observation 41 days.	1/100 calc. M.L.D. in total volume of 2 cc. injected intramuscularly into right hind extremity of each animal at 22 to 26 points. Extremity completely rigid on 6th day.
Feb. 27	S-5	22.27	0.045	1/8		
Feb. 27	S-6	16.81	0.025	1/8		
Feb. 26	S-4	17.1	0.07	1/4	Stiffness of left hind extremity began on 8th day; confined to posterior thigh muscles. Gait practically normal. Tendon reflexes normal. Period of observation 44 days.	1/36 calc. M.L.D. in total volume of 2 cc. injected intramuscularly into right hind extremity at 41 points. Extremity rigid on 5th day.
Feb. 26	S-3	10.6	0.083	1/2	Stiffness of posterior thigh muscles began on 5th day. Thigh became immovable on the pelvis. Gait nearly normal at all times. Tendon reflexes normal. Period of observation 44 days.	1/200 calc. M.L.D. in total volume of 2 cc. injected into each of four legs, intramuscularly, at 20 points, 35 days after the operation. All four ex-extremities rigid on 9th day. Died of intercurrent infection on April 22.

* The animals of this group, with the exception of S-3, are still alive and in good condition as this paper goes to press.

† The term "z-position" is used throughout these protocols to describe the position of an extremity tightly folded up against the body with hock, knee and hip joints completely flexed.

A detailed record of one of the animals is given in the following protocol:

Dog S-4. Male. Weight, 17.1 kg.

1935

Feb. 26, 11 A.M. Under ether anaesthesia the left sciatic nerve was exposed by the method described, and 0.07 cc. toxin 19b (representing 1/4 calculated M.L.D.) injected. A small amount of fluid was seen to escape after the needle was withdrawn. This was sponged away and after 10 minutes closure was effected. The dog was placed in a cage in order to restrict activity.

Feb. 27, 3 P.M. The patellar reflex of the left hind extremity is hyperactive. No trace of muscular stiffness. Gait is normal.

Feb. 28, 4 P.M. Entirely normal.

Mar. 1 Entirely normal.

Mar. 3 Entirely normal.

Mar. 5 Entirely normal.

Mar. 6, 9 A.M. In jumping out of the cage, the dog gashed his wound against the metal sheeting on the cage. A slight limp of this extremity was then observed. There is no limitation of movement and no change in the reflexes. The animal was released from the cage.

Mar. 7, 9 A.M. The dog has a slight limp, sparing the left hind foot. Mobility is unimpaired and the reflexes are normal.

Mar. 8, 12 N. Gait entirely normal. Reflexes normal. There is some resistance to complete flexion of the thigh against the abdomen.

Mar. 9, 10 A.M. Entirely normal except that resistance to complete flexion of the thigh on the abdomen persists.

Mar. 10, 12 N. Condition the same.

Mar. 11, 4 P.M. Condition the same.

Mar. 12, 11 A.M. The left thigh cannot be brought closer than 90° to the abdomen, even with the use of much force. The thigh is freely movable throughout the remainder of the arc (90° to 180°). Reflexes normal. Knee and hock joints easily flexed. The rigidity is completely localized to the posterior thigh muscles, that were first observed to be involved on March 6. There has been no extension of stiffness to muscles other than those poisoned by the escape of a small amount of toxin into the surrounding tissue at the site of injection.

Mar. 13 Condition the same.

Mar. 14 Condition the same.

Mar. 15 Condition the same.

Mar. 16 Condition the same.
Mar. 18 Condition the same.
Mar. 19 Condition the same. To test the susceptibility of this animal's muscles
11 A.M. to small doses of toxin, the dog was anaesthetized with ether and a 1/36 calculated M.L.D. of toxin (0.02 cc. toxin 22a in 2 cc. of extract broth) was injected into the muscles on the lateral aspect of the right hind extremity at 41 points. The dog was allowed to run in the paddock.
Mar. 20 Condition the same.
3 P.M.
Mar. 21 The right hind extremity is slightly stiff but the z-position can still be
11 A.M. enforced. Reflexes are normal.
Mar. 22 Condition the same.
4 P.M.
Mar. 23 Condition the same.
11 A.M.
Mar. 25 The right hind extremity is rigid in extension. The z-position cannot
10 A.M. be enforced. Reflexes are absent.
Mar. 27 Condition the same. Observations discontinued.
12 N.

Summary and Comments: Following the injection of 1/4 calculated M.L.D. in a total volume of 0.07 cc. into the left sciatic nerve of a dog, the animal remained entirely normal until the 8th day, when a slight limp appeared. On the 10th day there was some resistance to complete flexion of the thigh on the abdomen. This condition continued and slowly progressed until the 14th day, when the thigh could not be brought closer than 90° to the abdomen, after which time there was no extension or change in the stiffness for a period of 30 days, when observations were discontinued. The reflexes were normal, the knee and hock joints easily flexed, and the gait of the animal entirely normal throughout this period. Undoubtedly a very small amount of the toxin leaked out of the nerve and poisoned the muscles in close proximity, thus producing local tetanus in these muscles. This picture is in sharp contrast to that of the right hind extremity, which was injected intramuscularly on the 21st day with 1/36 calculated M.L.D. at multiple points to test the reaction of the animal to small doses of toxin. This extremity became slightly stiff on the second day and completely rigid in extension on the 5th day.

TABLE II

Group II. Showing results of the injection of 1/50 M.L.D. *into the left sciatic nerve of each of two dogs, in a total volume of 0.005 cc., when proper precautions were not observed to prevent leakage with subsequent poisoning of the surrounding muscles*

DATE	DOG NUMBER	WEIGHT	TOTAL VOLUME INJECTED	TOXIN IN M.L.D.'s	RESULTS	CONTROL EXPERIMENTS ON THE SUSCEPTIBILITY OF THE ANIMALS TO TOXIN
1935		*kg.*	*cc.*			
Mar. 11	S-9	14.55	0.005	1/50	In both animals slight stiffness began on the 7th day; confined to posterior thigh muscles. Motion of leg normal. Tendon reflexes normal. Period of observation 22 to 25 days.	Both animals died unexpectedly of intercurrent infections, S-9 twenty-five days and S-10 twenty-two days after injection. Intramuscular control injections were not made because of the early death of the animals.
	S-10	15.9	0.005	1/50		

A detailed record of one animal follows:

Dog S-10. Male. Weight, 15.9 kg.

1935

Mar. 11 11 A.M. Under ether anaesthesia the left sciatic nerve was exposed by the method described, and injected with 0.005 cc. toxin 19b (representing 1/50 calculated M.L.D.) No leakage was observed. The nerve was sponged lightly with moist gauze and the wound then closed. The animal was allowed to run in the paddock.

Mar. 13 11 A.M. Entirely normal.

Mar. 14 Entirely normal.

Mar. 16 Entirely normal.

Mar. 18 4 P.M. The dog spares the left hind foot a little in running.

Mar. 19 9 A.M. The sparing of the left hind foot is quite evident. The dog still bears weight upon it, but does not keep it off the ground. The z-position may be enforced, but there is some resistance to flexion of the thigh against the abdomen. Tendon reflexes are normal.

Mar. 20 3 P.M. Condition the same.

Mar. 21 Condition the same.

10 A.M.	
Mar. 22 4 P.M.	The left thigh can be moved only about 45°, and cannot be brought closer to the abdomen than 90°. Motion of the leg is normal, as are the tendon reflexes.
Mar. 23	Condition the same.
Mar. 25	Condition the same.
Mar. 28	Condition the same.
Mar. 30	Condition the same.
Apr. 1	Condition the same.
Apr. 3 9 A.M.	Dog found dead. Autopsy: A large bloody stool had been passed and was smeared over the buttocks of the animal. Fifty cc. of dark sanguineous fluid were found in the left pleural cavity, and about 75 cc. in the abdominal cavity. The intestinal wall was diffusely injected and hemorrhagic in spots.

Summary and Comments: The injection of 0.005 cc. of tetanus toxin (representing 1/50 calculated M.L.D.) into the left sciatic nerve of a dog produced no symptoms of central tetanus over a period of observation of 24 days, although local tetanus, beginning on the 7th day and confined entirely to the muscles near the site of the injection occurred. This was manifested solely by a marked resistance to flexion of the thigh on the abdomen. The tendon reflexes were normal and the motion of the leg was unimpaired. It should be remarked in this experiment that no precautions to prevent leakage of the toxin from the nerve were used, since closure of the wound was made immediately after the injection and the animal was allowed to run free in the paddock. Although undoubtedly only an infinitesimal portion of the toxin escaped from the injected nerve, this nevertheless sufficed to exert its specific effect on the nearby muscles. We infer that leakage of toxin from the injected nerve was facilitated by the dog's exercise in the paddock with others. Death from an intercurrent infection supervened before the susceptibility of the animal to the intramuscular injection of small doses of toxin could be tested.

TABLE III

Group III. Showing results of the injection of toxin into the left sciatic nerve of each of six dogs, without the subsequent appearance of either local or central manifestations of tetanus*

DATE	DOG NUMBER	WEIGHT	TOTAL VOLUME INJECTED	TOXIN IN M.L.D.'s	RESULTS	CONTROL EXPERIMENTS ON THE SUSCEPTIBILITY OF THE ANIMALS TO TOXIN
1935		*kg.*	*cc.*			
Feb. 20	S-1	15	0.005	1/48	Left hind extremity entirely normal. No central manifestations of tetanus. Period of observation 48 days.	1/50 calc. M.L.D. in total volume of 2 cc. injected intramuscularly into right hind extremity at 32 points. Extremity completely rigid on 6th day.
Mar. 27	S-11	17.27	0.012	1/50	Left hind extremity of each animal entirely normal. No central manifestations of tetanus. Period of observation 29 days.	1/100 calc. M.L.D. in total volume of 2 cc. injected intramuscularly into right hind extremity of each animal at 30 points. In each case, extremity rigid in extension on 6th day.
Mar. 27	S-12	14.55	0.01	1/50		
Apr. 1	S-13	18.18	0.013	1/50		
Apr. 1	S-14	13.63	0.01	1/50		
Mar. 6	S-7	21.37	0.02	1/17	Left hind extremity entirely normal. No central manifestations of tetanus. Period of observation 34 days.	1/50 calc. M.L.D. in total volume of 2 cc. injected intramuscularly into right hind extremity at 30 points. Extremity completely rigid on 5th day.

* The animals in this group are still alive and in good condition as this paper goes to press.

A detailed record of one of the animals follows:

Dog S-7. Male. Weight, 21.37 kg.

1935

Mar. 6, 11 A.M. Under ether anaesthesia the left sciatic nerve was exposed in the mid-thigh by the method described, and injected with 0.02 cc. toxin 19b, representing 1/17 calculated M.L.D. When the needle was withdrawn, a small drop of toxin was seen to leak back. This was sponged away thoroughly with moist gauze and the wound was closed after 10 minutes. The dog was placed in a cage in order to restrict activity.

Mar. 7 12 N. Entirely normal.

Mar. 8 12 N. Entirely normal.

Mar. 9 9 A.M. No abnormality of gait, mobility or reflexes. Dog has distemper Isolated from other animals.

Mar. 10 Condition the same.

Mar. 12 Condition the same.

Mar. 14 Condition the same.

Mar. 16 Condition the same.

Mar. 19 11 A.M. No further evidence of distemper. Released from cage and placed in paddock with other animals. Gait, reflexes and mobility entirely normal. Under ether anaesthesia the dog was injected with 1/50 calculated M.L.D. (0.02 cc. toxin 22a in 2 cc. extract broth) into the muscles of the right hind extremity on its lateral aspect at 30 points, to test his reaction to small doses of toxin.

Mar. 20 3 P.M. Entirely normal.

Mar. 21 11 A.M. Entirely normal.

Mar. 22 4 P.M. The right hind extremity is slightly stiff but the z-position can be enforced. Tendon reflexes normal.

Mar. 23 11 A.M. Considerable force is required to place the right hind extremity in the z-position. There is a constant tremor of slight amplitude of the entire extremity.

Mar. 24 10 A.M. The right hind extremity is rigid in extension. Patellar reflex absent. The other three extremities are normal.

Mar. 25 Condition the same.

Mar. 27 Condition the same.

Mar. 30 Condition the same.

Apr. 9 Observations discontinued.

Summary and Comments: The injection of 1/17 calculated M.L.D. into the left sciatic nerve of a dog, in a total volume of 0.02 cc., gave no symptoms of local or central tetanus over a period of observation of 34 days. A dose of 1/50 calculated M.L.D. in a total volume of 2.0 cc. injected intramuscularly into the right hind extremity at multiple points caused slight stiffness on the third day, progressing to complete rigidity of the entire extremity by the fifth day.

COMMENTS ON THE PROTOCOLS

Our experiments on the intraneural injection of tetanus toxin, that were made during the past year, fall into three groups, and we have

described all experiments made in that period—those that were completely successful (Group III) as well as those only partially so. The detailed protocols from each group have been taken at random, for one experiment is as good as another by way of illustrating our findings. Once we had learned to observe the precautions required for the successful performance of such injections, no further failures occurred.

Since we were using for these intraneural injections relatively small doses of toxin (that is to say, doses relatively small in respect to lethality), it seemed important to test the susceptibility of each animal's voluntary muscles to equal or even much smaller doses than were used in the nerve injections. This was done by injecting from 1/36 to 1/200 calculated M.L.D. into the muscles of the right hind extremity at multiple points. By this test all our animals appeared equally susceptible. Our method of calculating the minimal lethal dose for the dog is based on the figures found in a table compiled by Fildes (4), in which the dog is reported to be 150 times more resistant to tetanus toxin than the guinea-pig per gram of body weight, when the toxin is assayed in terms of minimal lethal doses for 350 gram guinea-pigs. In our numerous experiments on dogs for the past three years, we have found this figure entirely reliable, since a minimal lethal dose thus estimated has always killed the animal in four to five days.

In the first group of experiments, which were made before the importance of the necessary precautions was fully recognized, leakage from the injected part of the sciatic was not entirely avoided, and because of this leakage some degree of localized stiffness of muscles was noted, as described in our data. It should be emphasized, however, that even when partially developed local tetanus appeared, no portion of the toxin succeeded in reaching the central nervous system, as is shown by the fact that the motor cells of the spinal cord were never thrown into a hyperexcitable state. In a later paper it will be demonstrated that when a very small quantity of toxin is injected directly into either the cervical or the lumbar cord a very high degree of central excitability is induced.

In the two experiments described under Group II, in which as little as 0.005 cc. of toxin was injected, leakage of a very small portion of it

also occurred. The action of the poison on muscles is so marked that even an infinitesimal amount of the escaping toxin suffices to tetanize the few muscles with which it may come in contact. It will be shown in a subsequent paper that the response of a single voluntary muscle to the toxin constitutes the most delicate test for this substance now known to us. In the case of the two dogs in this group, because of the minute quantities injected (0.005 cc.), we were not sufficiently careful to exercise precautions which we had previously found necessary to prevent the accidental escape of the toxin from an injected nerve when larger volumes were employed. The very pronounced effect of hard labor in hastening and intensifying the action of the toxin was emphasized in experiments described in paper I. The clinical significance of such experiments will be obvious to those who know the history of tetanus and more especially of cases of the disease that were grouped by the older surgeons under the heading, *tetanus ambulans*.

An examination of the data afforded by the experiments described under the heading of Group III shows at a glance that here we had definitely learned just what care must be taken in order to insure complete success in making intraneural injections. One must have at hand, first of all, a powerful toxin so that the volume of the injected solution is not too large. The injection of an amount sufficiently large to cause bulging of the nerve would certainly be considered hazardous. It is surprising, however, that one can inject as much as 0.02 cc. of toxin solution, equivalent to 1/2 calculated M.L.D., by observing precautions that have been detailed in the section entitled "Method," without inducing either local or central symptoms of tetanus. It is also probably of importance to note whether or not the point of the injecting needle remains exactly in the center of the nerve trunk. Careful sponging of the nerve following the injection for ten minutes or longer, with the surrounding muscles adequately protected with gauze from absorbing any trace of the toxin that may leak back from the point of puncture, is also a necessary detail which cannot be neglected. The restriction of the activity of the animal for five or six days after the operation probably plays an important rôle in preventing the toxin from being squeezed out of the nerve by muscular massage.

In conclusion, then, we would emphasize that once one has learned

how to avoid leakage of intraneurally injected toxin, which manifests itself in due time by the inevitable appearance of local tetanus, one need never fail in making successful experiments such as those described under Group III. In none of the experiments, including those of Groups I and II where some leakage occurred, was there any evidence whatever that some of the toxin had been carried by the nerve trunk to the cord. The experiments of Group III prove that, when proper precautions are observed, an intraneural injection of toxin is exactly equivalent to a slow intravenous injection of an equal dose. This must be so, since the neural lymphatics, as was shown in papers I and II, cannot carry the toxin to the central nervous system, but only into the venous blood.

DISCUSSION AND SUMMARY

A study of our protocols shows that it is possible to inject otherwise strikingly effective doses of tetanus toxin into the sciatic nerve of a dog without the production of local tetanus in any of the muscles innervated by that nerve, or any other symptoms of tetanus anywhere in the body. Our experiments prove that when an amount of toxin, equivalent to or smaller than was used in such entirely negative intrasciatic injections, is injected intramuscularly or subcutaneously, either into a leg of a control animal or into the leg opposite to the one whose sciatic nerve has received the toxin, *local tetanus of a pronounced type always appears after the usual period of incubation.* There can be no doubt, then, that a given quantity of toxin that is entirely devoid of action when injected into a sciatic nerve manifests in a striking manner its specific localized action on muscles into which the poison has been directly injected.

It will be recalled, as was stated in our introduction, that, according to the accepted theory of our day, every intraneural injection of a moderate amount of the toxin into a mixed nerve, such as the sciatic, the obturator or the femoral, must inevitably be followed by local tetanus of all of the muscles of the corresponding extremity. It was believed that *voluntary muscles cannot be tetanized until the toxin has been conveyed by the injected nerve or nerves to the anterior horn cells of the spinal cord; and when these cells have absorbed the toxin, they pass into a hyperexcitable state and then respond more vigorously to kinaesthetic*

and other sensory impulses and send out a steady stream of motor impulses to the muscles of the leg in question, thus maintaining them for days and often months in a more or less rigid or contracted state. Believers in this purely central theory deny that localized, or partial, tetanus is ever caused, in any degree, by a local action of the toxin on muscle fibres with intact neural terminals.

They must now be asked to explain why a very minute amount of the toxin causes muscles into which it has been injected to become tetanized, while the intraneural injection of much larger amounts of it is entirely devoid of this action. The results of our experiments and the deductions that may legitimately be drawn from them may be stated in the form of the following propositions:

A. Tetanus toxin can be injected in doses of 1/50 to 1/17 M.L.D., and perhaps more, *into the trunk of a mixed nerve*, without exerting either a localized action on muscles that are innervated by it, or a central action. The toxin is not carried to the central nervous system by way of the peripheral nerves, but into the blood stream via their neural lymphatics.

B. Tetanus toxin can be injected *intravenously in even larger quantities than were used in A*, without inducing either a localized action on muscles or any symptoms of a central action. When such moderate amounts are injected intravenously, the blood stream fails to retain enough of the toxin to pass the brain-blood-barrier to reach the motor areas of the central nervous system. To reach these areas via the blood stream, larger quantities of the toxin than were used in A must be injected intravenously. Only blood-borne toxin can evoke the central, or reflex, convulsive type of tetanus.

C. Equal or even much smaller amounts of toxin than were used in A and B, with entirely negative results, tetanize muscles in a striking manner when they are injected into their substance. How small is the quantity required to induce complete rigidity of the muscles of an extremity is shown in experiments on animal S-3, Group I, in which the intramuscular injection of 1/200 of an M.L.D. into each of the animal's four legs caused all of them to become completely rigid. We know that this is not an isolated or single instance of the powerful local action of our toxin, since we have repeatedly injected such small quantities of it in many other experiments that will be described in our

next paper. In this particular animal (S-3 of Group I) the 1/200 M.L.D. which was sufficient to tetanize each leg is only 1/100 of the amount of toxin (1/2 M.L.D.) that had been injected into one of its sciatic nerves. In other words, the intraneurally injected 1/2 M.L.D. would have sufficed to make completely rigid 100 hind legs if injected intramuscularly, and yet this animal showed so slight a degree of stiffness of the muscles of the thigh that it could be detected only by passive manipulation. It is evident that the amount of toxin that escaped from the injected part of the sciatic in the mid-thigh must have been excessively minute, constituting probably less than 1/1000 of the originally injected 1/2 M.L.D.

D. Deduction in respect to the peripheral action of the toxin: Tetanus toxin acts very powerfully in a direct and specific manner on all voluntary muscles whose motor nerve terminals are intact. The action of the toxin is here as strictly peripheral as is that of curare. The deduction is justified, because the central action, postulated by the accepted theory as explaining observation C, is entirely excluded by the negative results of the experiments described under A and B.

Those who are familiar with the literature of tetanus will naturally inquire how our statement in reference to the direct action of the toxin on voluntary muscles (D) can be made to harmonize with the following well established facts: (1) When all of the nerves of a tetanized extremity are transected, its muscles will relax, immediately and completely, if their contracture has not persisted for more than a few days, but only incompletely, or not at all, if it has persisted to the final stage of complete rigidity; (2) and conversely, the muscles of an extremity, all of whose nerve trunks have just been transected, cannot be tetanized in the slightest degree by injecting them with an amount of toxin that will cause the muscles of a sound leg to become completely rigid and remain so for weeks or months. It has been repeatedly asserted that not only do these two observations strongly support the theory that the toxin induces local tetanus or muscle rigidity solely by virtue of its action on motor centers, but also that their physiological significance is such as to preclude even the possibility of a peripherally localized action of the toxin on voluntary muscles. Forty years ago such arguments carried conviction, but that is no longer the case, as can be shown by citing more recent phar-

macological experiments to the contrary. A year ago we made new experiments, that lead us to believe that when nerve transection experiments, like those referred to, are properly studied throughout their entire course by newer methods, they support rather than invalidate our belief in the peripheral action of the toxin. Our studies in relation to this and other points that furnish experimental proof of the correctness of our position will be reported in later papers.

When all of the data that have been given in this paper and in two preceding communications, together with the deductions that are drawn from them, are taken into consideration, we can only conclude that in so far as the toxin is absorbed by the haemal and more especially by the lymphatic capillaries at the seat of the natural infection in tetanus, it can be carried to the various tissues of the body, including those of the central nervous system, only by way of the arterial blood stream. Its distribution by lymphatic networks to muscles lying near the seat of infection or at a distance from it will be discussed at greater length in our next communication. It may be emphasized again that, in believing that water-soluble and locally non-destructive substances like tetanus toxin are not carried to the central nervous system in peripheral nerve trunks, we are confining ourselves to such observations with these substances as have been described in this and in our preceding communications. We have not concerned ourselves in our present studies with the manner in which living viruses are believed to migrate toward the central nervous system in certain cranial nerves—the olfactory and optic nerves. Nor are we concerned here with the manner in which dye stuffs or other agents, dissolved in such water-insoluble organic solvents as benzene, dekalin, turpentine and chloroform, move along the nerves of dead animals when such solutions are used for intraneural injections in anatomical studies (5).

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STUDIES ON VIRUS PROBLEMS

II. Cultivation Methods. The Development of Antibodies, in Particular, the Antiviral Body[1]

J. C. G. LEDINGHAM

Director, Lister Institute, London

In my first lecture I dealt with some of the very intriguing problems that are encountered in the attempt to unravel the nature of the interactions between the virus and the tissues of the host. The study of mingled resistances and susceptibilities to viruses residing in the various tissues of diverse animal species has at any rate made temporarily possible the securing of crude virus material of service in practical immunisation, and at the same time it has greatly extended the field for histological and other studies of virus adaptations. It should not be inferred, however, that the blanks in our knowledge of the intimate interactions, particularly the earliest interactions, between host and parasite are any greater in the virus group than among the ordinary bacterial pathogens, and accurate knowledge on this subject is yet sadly lacking. The great desideratum for future successful advance in our knowledge of the biological nature of viruses and also for advance and improvement in prophylactic methods against virus infection must be the further development of cultivation methods *in vitro*. To me it seems one of the most pressing problems in virus research. In introducing this subject I would say that it seems to me an unfortunate circumstance that more workers endowed with patience and perseverance (for it is tedious and tantalising work) are not forthcoming to devote themselves to cultivation work. Not so long ago pleuropneumonia was hailed as the shining example of a virus whose filterability did not preclude its cultivability on purely artificial media. Its place among the viruses was unique in consequence and one heard the remark, "If pleuropneumonia can be

[1]Lecture II (abridged) of the Herter Lectures, The Johns Hopkins University, Baltimore. Delivered December 6, 1934.

cultivated on artificial media, why not also this or that other virus?" The example is good and I would retain it as a lodestar, but, alas, neither pleuropneumonia nor its sister organism agalactia is entitled to a place among the viruses and in the textbooks they must now be banished to some special compartment among the bacteria. And why? Because though a filterable spore-like granule can reproduce the culture, that culture has a polymorphic and frankly microscopic morphology which inevitably precludes it from entry into the virus group. I need not go into the curious history of this organism and the strange and contradictory views that have been held about its cultural elements. I have dealt with this in a recent contribution to the study of its colonial growth. It is fair to say, however, that there has been throughout a lack of enterprise to apply to its study methods of no great complexity which might have settled its position many years ago. Critics who had not personally studied this organism were reluctant to accept as parts of a genuine cycle those strange morphological elements which Bordet in 1910 described in serum-broth cultures. They preferred to regard them as artefacts. Barnard's study of this organism, in 1925, by ultra-violet light photography, following the similar study of Frosch, also introduced unnecessary confusion in that he completely failed by this method to assign to this organism anything more in the way of structure than minute granules and spheres. It is true certainly, that the organism has weak affinity for stains and possesses elements of plastic nature which are readily distorted. Consequently, special technique must be elaborated for the fixation *in situ* and staining of growths on solid media, and for the investigation of liquid growths in thinly spread films from their earliest stages onwards. My own study of pleuropneumonia and agalactia in colonial growth was made by the use of an impression technique, while a later study by my colleague Miss E. Klieneberger was conducted by a modified agar-fixation method which yielded accurate pictures of the colonial elements with little or no distortion. The growth cycles as revealed by these two methods differed only in unessential details (See *J. Path. Bact.*, **37**, 393; **39**, 409). An interesting recent development of these studies on pleuropneumonia and agalactia has been the demonstration by Miss Klieneberger of the constant presence in cultures of *Streptobacillus moniliformis* of pleuropneumonia-like

symbionts which she ultimately succeeded in separating from the host and cultivating in pure culture (See *J. Path. Bact.*, **40**, 93). I mention this work here because of its bearing on the question of saprophytic viruses and on the theory of alleged filterable growth-phases in bacteria as the origins of viruses. If a culture of *S. moniliformis* were filtered and by a fortunate circumstance a culture of pleuropneumonia-like elements isolated from the filtrate, the inference might be drawn that *S. moniliformis* possessed an independent filterable growth-phase of entirely different morphology from that of the parent culture. Such inference would, of course, in this particular case be entirely erroneous. While, therefore, it may now be frankly admitted that pleuropneumonia and agalactia have no right to a place among the viruses in view of their frankly bacterial nature when cultivated on artificial media, so simple as serum agar or serum broth, it is not to be inferred that morphological change in the course of growth of the elementary bodies that probably constitute the infective agents in all viruses may not be shown to occur as knowledge advances. I have already alluded to the case of the Psittacosis bodies in this connection.

Now to pass to the great mass of what we are agreed to call genuine viruses and their capacity for *in vitro* development. I may say at once that with the one exception of the virus of vaccinia which my colleague Eagles has claimed to be able to cultivate in the absence of living cells, a claim, however, which has not yet received support from the few workers who have made the attempt to repeat his work—there is no evidence from cultural experiment that viruses are able to multiply *in vitro* apart from contact with living but not necessarily growing cells. Early efforts to cultivate vaccinia virus in the presence of tissue cultures were for the most part vitiated by the difficulty of obtaining bacteria-free seedings, and it may be said that modern work on this subject dates from Parker's and Parker and Nye's successful efforts (1923–1925) starting from vaccine-infected rabbit testicle—a source of bacteria-free virus first employed by Noguchi. These workers employed the intradermal method of test in the rabbit—a test which has greatly facilitated all work of this kind where estimates of potency must be made as accurately as a biological test will allow. Carrel and Rivers, in 1927, adopted chick embryo tissue with fowl plasma—a method which has shown itself fairly catholic in

its application to virus growth. A considerable simplification of the medium for vaccinia cultivation was made by the Maitlands, and by Maitland and Laing, by the substitution of freshly minced fowl or rabbit adult kidney in place of chick embryo tissue, together with Tyrode and fresh rabbit serum. This method was in a sense a challenge to convinced believers in the intracellular parasitism of viruses because, if not definitely stated, it was implied that the adult kidney cells in the medium underwent, in the course of incubation, fairly rapid autolysis. The method has been successfully used for a number of viruses, as I shall indicate, and the question early arose how far the multiplication that took place was attributable to the continued presence of surviving cells. It was pointed out by Rivers and Muckenfuss that the capacity of these kidney cells to proliferate when transferred to an appropriate medium was present even after 5 or 6 days' contact at incubator temperature with the virus. The implication, therefore, was that for growth of virus the presence of living cells was necessary, though not their actual proliferation—a fact which had been previously noted by Eagles and McClean in their experiments with the ordinary tissue culture method.

Once we have mentioned that certain well known viruses can grow in ordinary tissue culture or by the Maitland method, it would seem almost superfluous to catalogue the other viruses which have yielded to similar methods. I would just mention some outstanding cases which illustrate certain points of scientific interest. The tissue culture method with chick embryo has been successfully applied to fowlpox cultivation, as might have been expected. With the virus of foot and mouth disease, however, the Maitlands found that the catholicity of chick embryo tissue broke down and that growth of virus occurred only when the tissue supplied came from the hairy skin, pads and tongue of embryo guinea-pigs, thus confirming the well known susceptibility of foot and mouth virus for proliferation in these regions in the adult guinea-pig.

According to Haagen and Theiler, yellow fever virus multiplies best with minced chick embryo; virus III grows readily with finely minced rabbit testis and heparinised rabbit plasma. Andrewes also used successfully the Maitland technique with rabbit serum as for vaccinia and the characteristic intranuclear inclusions here made their appear-

ance. A study of the growth of this virus was also made by Topacio and Hyde of Johns Hopkins University. They, in contrast with Andrewes' result, got evidence of tissue immunity, in that inclusions failed to develop in experiments with washed immune tissue. Andrewes had found such tissue readily infectible.

Herpes simplex has also been readily grown *in vitro* with rabbit testis and rabbit plasma and the characteristic intranuclear inclusions have been demonstrated in the cultures.

Fowl-plague was found to multiply only in the presence of avian tissue, mammalian tissue proving quite refractory.

While an ever increasing number of viruses have yielded to efforts to secure their multiplication *in vitro* in the presence of susceptible cells, there remain certain outstanding viruses, particularly the strictly neurotropic ones like rabies, which have, so far, resisted cultivation attempts *in vitro*. At any rate it is fair to say that the evidence so far brought forward of successful *in vitro* growth is not entirely conclusive.

I must make reference here to typhus fever though it is doubtful if this virus, whether we consider the classical type or the many varieties which research of recent years has revealed in many countries, is to be reckoned among the filterable viruses. If, as is now generally admitted, the Rickettsia bodies represent the infecting agencies in classical typhus fever, Rocky Mountain Spotted Fever, Tsutsugamushi, etc., their size would probably explain the difficulty experienced in securing takes with filtrates so that, strictly speaking, typhus fever and its congeners would not come quite into the category of filterable viruses. Nevertheless, the problems of their cultivation *in vitro* are those of the strict viruses. Thus Nigg and Landsteiner have employed successfully the Maitland method as used for vaccinia, the tissue being the minced normal tunica of half-grown guinea-pigs with Tyrode and serum. The passages were carried through many generations with no loss of virulence. The fact that Rickettsia can be cultivated in this way has facilitated the investigation of one of the most perplexing problems in serology—the meaning of the Weil-Felix reaction. My colleague Felix in recent years has not lost sight of this problem and he has materially contributed to our knowledge of this reaction and its diagnostic importance in the many typhus-like dis-

eases which have come to light in recent years in many parts of the world. It would seem from recent work in Boston that Proteus X strains and Rickettsia bodies do share some common antigen, but as yet it is idle to speculate how far, if at all, these two rather widely different organisms form part of a single cycle. It would be a great relief if they did and we got rid of what has been for many years a serological puzzle.

Before I summarise this subject of *in vitro* cultivation of viruses I must refer to my colleague Eagles' work on which he has been engaged now for some years, with his collaborators. He has published now a succession of papers in which he demonstrates the growth of vaccinia in media claimed to be free of living intact cells, but containing the cell substance or cell granules. Criticisms have been made on the ground that the centrifugalisation of the kidney tissue has still left an occasional living cell or cells which have enabled growth of virus to take place. When one considers what a centrifuge working at 10,000 r.p.m. can accomplish in the way of depositing a suspension of cells, and when one, after careful examination, by various methods, of the supernatant granular material, is able to persuade oneself that probably no intact cells are left, it would seem at any rate unreasonable to attribute the growth which takes place to the faint possibility of a few residual cells surviving. It would reduce, to my mind, the alleged living cell requirement for virus growth to an absurdity. However, until this work has been confirmed, and it has been independently shown that vaccinia virus can obtain its energy for growth from the cell constituents, one has to accept the present rather unfortunate deadlock that viruses cannot be grown apart from living cells. As I have said, virus culture work is tedious and tantalising at the best and accommodation to growth in cell-free media particularly so, as Eagles has found. Good survival in the first passage is easy, but in further passages there is a very definite tendency for growth to drop off. Sometimes it may be necessary to go back to the beginning 5 or 6 times before it is possible to get beyond the first passage, when as a rule the chances are that it goes on. Eagles' cell-free method I may just summarise. Both rabbit kidneys are put through a mincing machine. The minced material is moistened with 1 c.c. of 9 per cent saline to break up the cells. It is then brought

back to normal saline, giving in all 10 c.c. in normal saline. This is now spun for half an hour at 10,000 r.p.m. The supernatant is taken off down to within $\frac{1}{4}$-inch of the deposit with a fine capillary pipette. Thus about 5–7 c.c. of extract are obtained. To this is added 4 c.c. of normal rabbit serum and 1 c.c. of virus (being the deposit of centrifuged vaccinia filtrate and containing only elementary bodies), 8 c.c. of Tyrode is added, to a total of 20 c.c., so that the virus is always in a concentration of 1 in 20. The mixture is now distributed in 2 c.c. amounts in Carrel flasks. Maximum yields of growth are obtained usually on the 4th or 5th days of incubation.

If the cell granules are centrifuged down completely at 15,000 r.p.m. no growth of virus can be obtained with the supernatant.

If we set aside Eagles' claim[2] which is not yet confirmed but which, if confirmed, would undoubtedly stimulate research in a field which seems to have reached a dead end, what have we learned of the biological nature of viruses from these cultivation experiments except that they emphasize the necessity for the presence of whole living cells and that in *in vitro* culture the viruses exhibit to some minor extent the cytotropisms that characterise them in the natural infection? Beyond the fact that they require living cells in which presumably they multiply—and indeed the *in vitro* development of inclusions would support this view—we have learnt little of their essential metabolism. Work is required on the respiratory activity of cells in these cultures and on the modifications of such respiratory activity by the simultaneous growth of viruses in association with them.

Maitland claimed that the virus of vaccinia ceased to grow after the tissue ceased to respire. Yet his own experiments show that some of the best increases in growth of virus took place after the 3rd or 4th day, when the tissue ceased to respire, so that the matter is by no means clear. One might also cite the fact that Dochez and his collaborators, in their experiments on the growth of the common cold virus, used chick embryo under a vaseline seal, when presumably there was no free respiration of the cells.

The interactions, therefore, between virus and living cell may be

[2] See *Brit. J. Exp. Path.*, April, 1935.

said to be practically unknown so far, but the position is by no means hopeless if determined workers, undeterred by the inherent difficulties of the subject, are prepared to devote their time to it. What is required is an intensive study of the growth of one virus—it matters little which—under conditions held to be essential for its growth. Vaccinia and fowlpox would be eminently suitable in view of the fact that the Paschen and Borrel bodies, which we believe to constitute the virus, can be detected with fair ease in these cultures. Both Eagles and myself and Paschen have had no difficulty in demonstrating enormous numbers of elementary bodies in successful cultures at late periods of passage. Growth should be followed by improved technique illustrating the behaviour of the elementary bodies at all stages.

ANTIBODIES, PARTICULARLY THE ANTIVIRAL BODY

I wish to conclude this lecture with some comments and observations on the antibodies that are developed in response to virus infections, whether such infections are of the frank clinical order or of the subclinical or inapparent type. That an antiviral body exists, capable of neutralising the action *in vivo* of a virus when simultaneously introduced with it, was first shown in 1892 by Sternberg in the case of vaccinia and consequently antivaccinial serum is the prototype of all the many antiviral sera since shown to characterise infection with, or recovery from, the great majority of viruses. Whether such sera are to take any prominent place in the therapeutic armamentarium against virus disease is as yet difficult to say, but certain features of their action and certain features of virus action would seem at the moment to render their use in therapy of probably minor importance. In prophylaxis of virus disease, we know they have for many years proved their value, sometimes when administered alone, as in the prevention of measles, but more often in conjunction with the virus whose action it is designed to mitigate. For some years there has been considerable speculation as to the mechanism of action of such antiviral sera. I have had a good deal of experience in the testing of antivaccinial sera prepared in the rabbit and the horse, and in my early work on the histology of the dermal response to vaccinia in the rabbit I was able to show that, when the response was prevented by antiviral serum injected in conjunction with the virus, any action on the local

reticulo-endothelial tissue, particularly the endothelium of the small vessels, was in abeyance, thus suggesting that the main attack of the virus was probably directed against the local reticulo-endothelial system. Partially neutralised mixtures often yielded residual pale non-oedematous nodes consisting of dense infiltrates of adventitial cells perivascularly arranged. I feel, however, in view of the importance of understanding the exact mechanism of neutralisation of virus action *in vivo*, that the histological method may throw light on the matter only if inoculated spots are removed at very short intervals after inoculation of the mixture of virus and immune serum. This has still to be done. This is all the more necessary, as we have since learnt how rapid is the attack of virus on susceptible tissue, that in fact, if immune serum is superposed 5 or 10 minutes later on a virus-infected skin site, it is of little or no avail in effecting neutralisation. As examples of this rapid fixation of virus to susceptible tissue I may cite some experiments from a paper published a few years ago (*Brit. J. Exp. Path.*, **12,** 357) on this subject. A series of rabbits (in duplicate) were scarified with vaccinia and also inoculated intradermally with this virus. One couple was given antivaccinial serum intravenously 24 hours before the virus inoculations, another simultaneously, while the third and fourth couples received the serum 24 hours and 48 hours, respectively, after the virus. The cutaneous responses were absent only when the serum was given 24 hours before or simultaneously, whereas the intradermal responses were in no instance abolished though greatly reduced in intensity in comparison with those in the controls.

When a similar test was made with the generalising neuro-testicular strain of vaccinia virus given intravenously, the simultaneous intravenous injection of antivaccinial serum abolished the usual pyrexia, and visceral lesions were absent except for some few pocks on the pleural surfaces of the lungs. Even these were in abeyance when the antivaccinial serum was given two hours before the injection of virus. Injection of serum at the early pyrexial stage in another set of rabbits did not appear to be followed by significant amelioration of the condition. It would seem, therefore, that the field of therapeutic use of antivaccinial serum may be rather limited, but one cannot directly argue from experiments in which a highly susceptible animal is over-

whelmed at once with a massive dose of the virus, that antiviral serum may not serve a useful purpose in human therapy, *e.g.*, in the treatment of virulent smallpox. Some physicians are prepared to accept the view that convalescent poliomyelitis serum is of value therapeutically in the preparalytic stage and act accordingly, but probably a majority believe that no evidence so far has been forthcoming that such serum exerts any therapeutic effect whatever. In any case accurate controlled statistics in support of the procedure are not yet available. I believe, however, that there is a field for antipoliomyelitis serum as a prophylactic for contacts though here again it will be difficult to get the necessary statistical controls.

A recent serious outbreak of poliomyelitis in Denmark, in which extensive prophylactic use has been made of antipoliomyelitis serum from the horse, may possibly afford some evidence for or against its value in such circumstances. This antipoliomyelitis serum prepared in the horse has high neutralising value—considerably higher than that of human convalescent serum—when tested on monkeys. To return to the mechanism of action of such sera. When these sera are fractionated and we have experience in this connection both of antivaccinial serum and of antipoliomyelitis serum prepared in horses, the effective antibody is present both in the euglobulin and the pseudoglobulin, and in the former the concentration is probably higher, but this higher concentration is more than compensated by the higher absolute amount of pseudoglobulin in the natural serum.

So far then as chemical fractionation of the antiviral serum goes, the antiviral body appears to behave like many other antibodies, antiviral and antibacterial. What precisely is the antigen to which it is the antibody? How does it behave in contact with the virus *in vitro?* Can it be fixed by the virus and, if so, how close is the union? We know that for *in vivo* neutralisation the mixture of virus and immune serum needs no previous digestion *in vitro*. The view I have always entertained, though as yet it cannot be proved, is that the virus, when digested with the immune serum *in vitro* or simply inoculated in mixture directly with the immune serum, is sensitised or opsonised and that the phagocytes *in vivo* do the rest, the susceptible cells being thus spared from attack. Agglutination of the elementary bodies *in vivo* may also prove a most important virus-localising factor. That

the union of virus and antiviral serum is weak and easily dissociable, *e.g.*, by dilution of a neutral mixture, and only becomes irreversible after long digestion, has been made at least very probable by the work of Andrewes, Bedson, Schultze and others on vaccinia, herpes febrilis and poliomyelitis. It has been held, moreover, that the fact that mixtures of virus and antiviral serum, which are neutral or produce no action when injected into one tissue, produce infection in another tissue, militates against any real union between virus and antiserum *in vitro*. This phenomenon, however, may be due simply to differences in the nature and amounts of the reticulo-endothelial tissue of the site into which the mixture is injected. Though it has been shown by experiments *in vitro* that the occurrence of inclusions (*e.g.*, herpes) is prevented by immune serum and consequently in the apparent absence of leucocytes, there is little doubt from experiments by Wilson Smith and Fairbrother and others that the leucocytes in presence of immune antiviral serum have a much enhanced power of disposing of virus.

Sabin, working at the Lister Institute, has recently completed a new study of this problem which has led him to the conclusion that virus and antiviral serum (neutralising serum) do not combine. For this work he has made extensive use of the high speed centrifuge. When he mixed known quantities of virus (as Berkefeld filtrates) and immune serum or normal serum, left them to incubate for varying periods and then centrifuged the mixtures, he found on reconstituting the sediments and testing them for infectivity that there was no evidence of combination. This occurred both with B virus and vaccinia. Then he mixed vaccinia and antivaccinial serum and minced testes and Tyrode, left the mixture in the cold and then centrifuged. The virus was not destroyed, but was present both in the fluid medium and in the washed cells. He then put together normal leucocytes, fresh normal serum and vaccinia and incubated at 37° with shaking. The virus was fixed by the leucocytes, which became highly infectious, but there was no evidence of destruction of virus.

On repeating this experiment with immune serum the washed leucocytes also contained virus, but not so much as in the normal serum mixture. When normal leucocytes were mixed simply with immune serum, kept at 37° for 4 hours and then washed and frozen and thawed

10 times, these leucocytes were found to have fixed antiviral substance, as shown by their protective power against added virus.

The general conclusion he arrives at from all the various experiments is that the protective substance acts upon the tissue, rendering it refractory to infection and unsuitable for multiplication of virus. The protective substance is fixed by the tissue, but both the fixation and the effect are reversible. The leucocytes do not, in his view, play a very important rôle in the mechanism of immunity.

This is a hard saying and it would appear that a sort of impasse has been reached. I do not, however, feel disposed to give up the idea that some sort of union takes place between the virus and the neutralising antibody. To think otherwise would be to give up the idea that the antiviral body is a real antibody at all. If the virus is not its antigen, then the antiviral serum must find an antigen in some portion of the cell, or some surface cell product which is so altered that the virus fails to penetrate the cell. We shall have to await further experiment, I am afraid, before expressing any dogmatic views as to the apparent lack of combination between virus and antiviral serum. When Sabin's experiments are published[3] it will be time enough to consider the problem afresh and perhaps to formulate other interpretations of the data more in accordance with what we know of antigen-antibody reactions generally.

While the mechanism of neutralisation of virus action by antiviral serum is still obscure and not readily explained on a basis of sensitization of virus involving some form of antigen-antibody combination, other interactions occurring *in vitro* between virus and the corresponding immune serum such as precipitation and complement fixation, have been fairly generally accepted as genuine antigen-antibody reactions. Where crude virus extracts used as antigens in such reactions have been liable to contamination with bacterial antigen and the corresponding immune serum has consequently come to contain antibody not only for the virus but also for the contaminating antigen, valid criticisms have been directed against the specificity of the reactions. In the case of the flocculation reaction taking place between vaccinia virus and antivaccinial serum (Gordon, Tulloch

[3] See *Brit. J. Exp. Path.*, 16, 70, 84, 158, 169.

and Craigie) this criticism has been fully met by the use of bacteria-free virus obtained from infected organs. There would seem no reason, therefore, to doubt that, in this reaction as also in the complement-fixation reactions with herpes labialis, dog distemper, foot and mouth virus etc., and their corresponding antisera, genuine antigen-antibody combinations are playing a part. It is, however, highly desirable that in serological reactions between viruses and their immune sera, the purity of the virus antigen should be the greatest possible and in my third lecture I hope to refer to serological investigations with the elementary bodies themselves.

RELATION OF THE CUSHING SYNDROME TO THE PARS INTERMEDIA OF THE HYPOPHYSIS

W. G. MacCALLUM, T. B. FUTCHER, G. LYMAN DUFF AND READ ELLSWORTH

The Johns Hopkins University, Baltimore, Md.

Submitted for publication May 28, 1935

The recognition by Harvey Cushing (1) of the symptom complex described by him in 1932, and its dependence upon the presence of a small tumor of a peculiar type in the hypophysis, is a lasting contribution to medical knowledge. Various tumors had been described before this and Erdheim and Nothdurft had especially studied basophile adenomas, but their effect in producing the extraordinary combination of profound changes in the body had not been realised. Since Cushing's correlation of these changes—extreme obesity of face and trunk with broad cutaneous striae, osteoporosis, hypertension, diabetes and cessation of gonad function,—there have appeared in the literature numerous papers describing quite similar cases. Indeed, in several instances in which the symptoms were described without finding a tumor in the hypophysis, further search at Cushing's suggestion has revealed the presence of a small basophile adenoma which had been overlooked.

But there are also cases in increasing numbers in which the same, or nearly the same, set of changes is found to be dependent upon a tumor of the adrenal cortex, so that in each case the diagnosis must rest upon the exclusion of an adrenal cortical tumor because the hypophyseal tumor is so small as to produce no changes visible by x-ray examination. Removal of such an adrenal tumor has resulted in several cases in the return to normal of all the changed conditions. So, too, radiation of a tumor of the hypophysis has in some instances caused the disappearance of all the abnormalities (Jamin (2)). It seems, therefore, that there must be some close relation between disturbances caused directly by the overaction of the adrenal cortex and

those resulting from the hypophyseal tumor which may perhaps in turn stir to over-activity the adrenal cortex.

Despite intense study by many experimenters, these relations are still in a nebulous state. It is, of course, known that hypophysectomy causes atrophy of the adrenal cortex and that regeneration follows the implantation of tissue of the anterior lobe, or injection of an extract, but in all such results the question arises as to which cells are responsible for this control of the adrenal cortex.

It seems hardly necessary to review again the literature on these numerous cases, since it has been done so many times since Cushing's paper in 1932, but for convenience a list of references may be given. The occasion of this paper is the study of a typical instance of the Cushing syndrome in a woman of 25 years, with typical anatomical changes at autopsy. The case was as follows (Dr. Futcher):

An unmarried Polish Jewess, aged 25 years (J.H.H. Unit No. 59055) was first seen in the outpatient department on November 7th, 1934, complaining of increased weight, change in her appearance, and amenorrhoea. Her health had been good previous to the onset of her present illness one year ago. Menstruation was regular up to that time when the flow became more scanty and ceased two months ago. Her weight which averaged 170 pounds, increased to 220 pounds but had fallen recently to 192. She realised that her facial appearance had changed and noted the development of broad reddish-purple lines over her shoulders, chest and abdomen. Nine months ago there was oedema of feet and legs up to her knees which still occurs at the end of the day. The dyspnoea, palpitation and ready fatigue she attributed to her increased weight. About 7 months ago she developed polyphagia and polydipsia and said that she would drink two gallons of water daily. There was also very marked polyuria. Her hair began to fall out and she complained of numbness and tingling of hands, dizziness, a feeling of body warmth and night sweats.

Physical examination. Height, 5 ft. 6 inches. Weight, 192 lbs. (average normal weight for this height should be 130 lbs.). The fat is especially over face, chest and abdomen. The face is very red. Numerous reddish-purple striae over the shoulders, sides of the chest and particularly over the abdomen, where they run vertically and are 1–1.5 cm. broad. They extend down over the iliac crests and the thighs and legs have a curious mottled redness. There is no marked hirsutism but the hair on head and pubis is normal. Eyes seem normal. Thyroid not enlarged. Lungs clear on percussion and auscultation. Heart slightly enlarged, aortic second sound accentuated. Blood pressure 210/150. Abdomen shows only the great adiposity—no abnormality in size or position of the organs can be determined.

The spine shows an exaggeration of the normal upper dorsal curve. The joints are, in general, normal.

Roentgenograms at this time showed a slight infiltration of the apex of the right lung. Sella turcica normal in size and outline. Bones show evidence of demineralization.

Wassermann negative. Blood N.P.N. was 32 mgm. per cent, but unfortunately there is no record of a blood-sugar determination. Urine pale, turbid, acid, with large quantities of sugar and albumin but no ketone bodies. Microscopically, a few hyaline casts and leucocytes. Blood count: R.B.C. 6,050,000; W.B.C. 18,000; Hb. 114 per cent.

The finding of osteoporosis of the vertebrae, as well as of the skull, although there was no collapse of the bodies of the vertebrae, the high red count and high haemoglobin, the glycosuria and the obesity and cutaneous striae, seemed convincing evidence that this was a case of Cushing's syndrome. The patient consented to enter the hospital for further study and treatment and was admitted on November 13th, 1934. It was intended to investigate more carefully her blood counts, blood pressure, basal, carbohydrate and calcium metabolism and to determine whether the urine contained any anterior pituitary hormones.

On November 18th the red corpuscles were 5,725,000, Hgb. 14.5 gms. or 109 per cent. On November 15th the blood showed the following: N.P.N. 40 mgm. per cent; fasting blood sugar 255 mgm. per cent; calcium 11.2 mgm. per cent; phosphorus 4 mgm. per cent. Basal metabolic rate,—6. The urine showed no diacetic acid but the 2700 cc. excreted on that day contained 3 per cent of sugar with a total output of 81 gms. Insulin was given but the urine never became free of sugar.

Dr. Ellsworth attempted to demonstrate the presence of gonad-stimulating hormones in the urine. The Friedman rabbit test showed no evidence of an ovulation producing factor (Prolan B of Zondek). Urine was then extracted by the Zondek method. The 75 per cent alcohol precipitate was thoroughly washed with ether and taken up in water (*i.e.*, the urine was concentrated ten times) and injected into immature mice (4 weeks old). A total of 2 millilitres in five doses (equivalent of 20 millilitres of urine) were injected into each animal. Gross and microscopic examination after 100 hours showed no changes in ovaries, uterus or vagina.

In addition, 10 millilitres of filtered, unconcentrated urine were injected daily for 3 weeks into two adult female rabbits. A third rabbit was injected with normal urine at the same time. These animals, being killed and examined, showed no changes in adrenals, thyroid or parathyroid when compared with the control. Examina-

tion of the blood of the patient for gonad-stimulating diabetogenic or adrenotropic hormones was prevented by her sudden death.

Gynaecological examination of the patient showed nothing, but in order to exclude the possible existence of an adrenal tumor it was decided to have pyelograms taken and this was done for the right kidney, showing no abnormality of the pelvis. Following this examination the patient's temperature rose on November 23rd to 103.8°F. The impression was that she had developed a pyelitis and generalised infection, and in spite of every effort to overcome this, she died on November 28th. Cultures from the bladder and from the blood on November 24th showed a growth of staphylococcus aureus.

A pyelogram of the left kidney was never taken owing to the development of acute complications on the right side. Considering the unfortunate developments following the taking of the pyelogram in this case and knowing as we do that patients with pituitary basophilism are very subject to infection, it seems very important that such examinations should not be carried out in the studies of these cases.

The *clinical diagnosis* was, therefore, Cushing's syndrome; diabetes mellitus; pyogenic infection of the right kidney; general septicaemia.

Autopsy No. 13960 (Dr. Duff). The report is abbreviated to give in detail only those changes apparently associated with the presence of the hypophyseal tumor.

The body is that of a very obese adult white female said to be 25 years of age but which appears to be nearer 40. Weight 90 kilograms, length 177 cm. There are pads of fat on the face, especially in the region of the parotids, and rolls of fat on the neck. Shoulders and upper part of the chest covered by thick layers of fat. The abdomen also shows a layer of fat which in the midline is 6 cm. in thickness. The legs are much thinner. Where the fat is most abundant there are broad striae in the skin. There are none of these on face or neck, but they are numerous over the upper chest, especially in the axillary folds and beneath the breasts. Many are disposed in transverse direction in the skin of the abdomen where they measure 2–5 cm. in length and vary in width from a few millimetres to 1.5 cm. They were bright red during life but are now pale. Others over the lower abdomen are longer and curved and arranged more in a vertical direction. The distribution and quantity of hair are not abnormal. The external genitalia appear normal, the clitoris is not enlarged.

The peritoneal surfaces are smooth. The abdominal and pelvic organs appear normal but the right kidney is embedded in a firm mass of retroperitoneal fatty tissue. The thoracic and pericardial cavities show no abnormalities. No recognisable thymus is found in a great mass of mediastinal fat. The heart weighs 410 grams which seems remarkable since there was rather extreme hypertension.

The myocardium shows several abscesses and there are vegetations on the mitral valve (later found to contain great quantities of staphylococcus aureus). The lungs show numerous minute abscesses and a localised encapsulated caseous tuberculous area near the right apex. The spleen weighs 200 grams and presents the appearance of an acute splenic tumor. Liver weighs 2110 grams and is soft, but otherwise apparently normal. The pancreas shows some fat necroses in the pericapsular fat but is otherwise normal. The intestinal tract apparently is normal throughout.

Adrenals: The left adrenal, freed carefully from all fat, weighs 14.75 grams. The right adrenal is embedded in the inflamed fatty tissue at the upper pole of the right kidney and the fat could not be cleaned from it so thoroughly. It weighs 18 grams but it really appears smaller than the left adrenal and it is estimated that about 5 grams of this weight consists of fat. The adrenal glands are slightly larger than normal, the right measuring 4.5 cm. x 6 cm. and 3–4.5 mm. in thickness. The left measures 4 cm. transversely by 6 cm. vertically. Their external configuration is normal. On numerous cross sections the tissue appears normal except that the right adrenal shows a number of small abscesses scattered through its substance. The cortex is filled with bright orange yellow lipoid. There are no adenomatous nodules in the cortex and the distribution of fat is practically uniform throughout. The intermediate zone is thrown into relief, evidently more by the hyperaemia of this layer than by pronounced pigmentation (but when fixed it shows as a brown layer 0.5 mm. thick). The medulla is pale grey and the tissue appears to be present in normal quantity. The blood vessels show nothing remarkable.

The *left kidney* weighs 210 grams and measures 13 x 6.5 x 3.5 cm. Its surface is smooth and on section the cortex, pyramids and pelvis appear normal. The *right kidney* is embedded in a dense mass of fat rendered firm by an inflammatory process, and when the capsule is stripped off, the kidney still weighs over 400 grams. It is greatly enlarged and riddled with abscesses. The pelvis is dilated and its walls thickened by the inflammatory reaction. The ureter is similarly affected, although the bladder shows little change except about that ureteral orifice. The uterus is of normal size and shape, the endometrium pale and smooth. The Fallopian tubes are flexible and soft with delicate fimbriated ends. The ovaries are of normal size and shape and show normal appearance both externally and on section.

The thyroid is normal in form and size. No nodules are seen either externally or on the cut surface. The tissue is homogeneous and appears to contain a moderate amount of colloid. Four parathyroid glands are found in the normal positions. Their size is normal and they contain no gross nodules. The carotid sinuses are removed from both sides and show nothing remarkable. No accessory chromaffin tissue was found at any point.

Cranial Cavity: The skull measures 8 mm. in thickness and can be cut with a knife. The external and internal tables and the cancellous laminae are dis-

tinctly thinner than normal. The dura, venous sinuses, vessels at the base of the brain and the brain itself appear normal. The pineal gland is normal in size and shape.

The *hypophysis* was removed with the greatest care. Its size and shape are about normal although it is perhaps very slightly larger than usual. The anterior and posterior lobes are of the usual shape and their relative size is about normal. On the inferior surface of the hypophysis there is a small flattened mass of tissue measuring 7 x 7 mm. which projects for about 1 mm. from the lower surface of the anterior lobe. It extends to a point about 1 mm. from the posterior margin of the anterior lobe and does not come into close relationship with the posterior lobe. It is soft and pale grey or yellowish-grey and marked out from the tissue of the anterior lobe by this pale color and by its softness. One median horizontal section, made to allow proper fixation, is evidently above the nodule, for it does not show on the cut surface.

Study of the microscopical sections from the organs show in general the changes produced by an extremely intense and rapidly developed staphylococcal septicaemia arising from the infection of the kidney. These need not be further discussed. Otherwise the tissues in general show little change.

The *pancreas* shows in a section of the head some small areas in which the acini are dilated. Sections through the body and tail of the organ show many minute pancreatic ducts which are dilated and filled with inspissated secretion, and many show metaplasia of the lining epithelium to a squamous type. Corresponding with these obstructions there are groups of dilated acini and scarred areas containing only dilated ducts. The islets of Langerhans in these and other sections are present in the usual numbers and are of normal appearance. The fat necroses are old and encapsulated. Most of them occur in the fat around the periphery of the pancreatic tissue but a few are found in the substance of the pancreas. No hyaline arterioles are seen.

The *adrenals*, with our present inadequate methods of study, show little change. There is a fairly thick cortex in which the cells of the glomerular zone are not unduly large and in some places even appear shrunken. Those of the fasciculate zone are plump and possibly slightly increased in bulk. The reticular zone is hyperaemic but otherwise appears normal and the medulla, except perhaps for some small accumulations of round cells, is also normal.

Sections of the ovaries show many primordial follicles arranged along the superficial layers and a number of hyaline corpora albicantia but few, if any, ripening Graafian follicles.

The thyroid shows no adenomatous nodules and in general the tissue is uniformly made up of rather large acini filled with colloid and lined with low cuboidal or flattened epithelium. The four parathyroids appear normal in every particular, although the strands of cells are rather widely separated by fat tissue. Sections from the mass of fat in the anterior mediastinum show only small atrophied remnants of thymus tissue. The pineal gland shows no significant changes.

The skull in section shows a marked thinning of the inner and outer tables which are separated by cancellous bone in which the laminae are distinctly thinner than normal, although there is no evidence of any erosion by osteoclasts. The same changes are found in the laminae of the vertebrae.

Sections through the striae in the skin of the chest and abdomen show the corium stretched out so that it has become relatively thin with a loss of much of its connective tissue and nearly all of the elastic fibres. The overlying epithelium is drawn out into a thin smooth layer with the disappearance of its papillae. The widening of blood vessels, which must have produced the deep red color, is not recognisable in the section.

FIG. 1. ADENOMA EXTENDING INTO ANTERIOR LOBE OF THE HYPOPHYSIS. STAINED WITH MANN'S METHYL-BLUE-EOSIN STAIN

The *hypophysis* was fixed, as were the other tissues, in Zenker-formol and cut horizontally into a complete series of sections carefully numbered and kept in order. These sections were stained in many ways but all served well to show the relation of the tumor to the normal tissues. The tumor appears only in the sections of the inferior half of the hypophysis and at its greatest diameter measures only 6 mm. It is practically spherical and is embedded in the posterior portion of the anterior lobe projecting from the inferior surface

of the hypophysis where it is distinctly separated from the posterior lobe. Higher in the series of sections it extends backward, however, and although it does not at any point invade the posterior lobe, it does become continuous with the intermediate zone so that no line of demarcation can be recognised. On the other hand, it is sharply outlined against the tissue of the anterior lobe, the cells of which are compressed and flattened about it (Figs. 1 and 2).

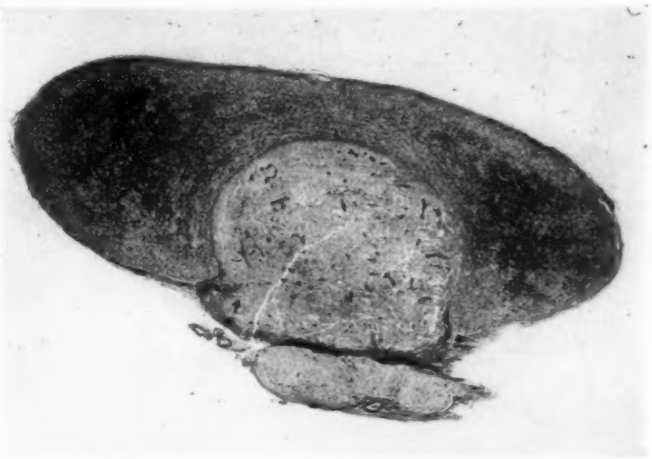

FIG. 2. ANOTHER SECTION STAINED WITH COPPER HAEMATOXYLIN SHOWING THE CONTRAST WITH BASOPHILIC CELLS OF THE ANTERIOR LOBE AND THE CLOSE RELATION WITH THE PARS INTERMEDIA

The adenoma is composed of cells of fairly uniform size and shape with a rather characteristic radial arrangement about the numerous minute vessels which branch everywhere through it (Fig. 3). These cells are elongated, almost columnar in form, and are best preserved as they lie with their long axis radiating from the blood vessels. Apart from this arrangement they become rather disordered and those at the greatest distance from the blood vessels tend to become necrotic. The groups of necrotic cells are small and irregular. There is very little supporting framework. In sections stained with haematoxylin and eosin the tumor cells appear as cubical or cylindrical

forms with rather small round nuclei placed toward one end of the cell, and where they are arranged around a blood vessel the nucleus is at the end of the cell nearest the blood vessel. The cytoplasm appears homogeneous and stains a pale orange-pink. But when stained especially to demonstrate granules with such stains as pyrrhol blue and eosin, water blue and eosin or Mann's methyl blue and eosin, as recommended by Kindell (3), or with some of the other stains

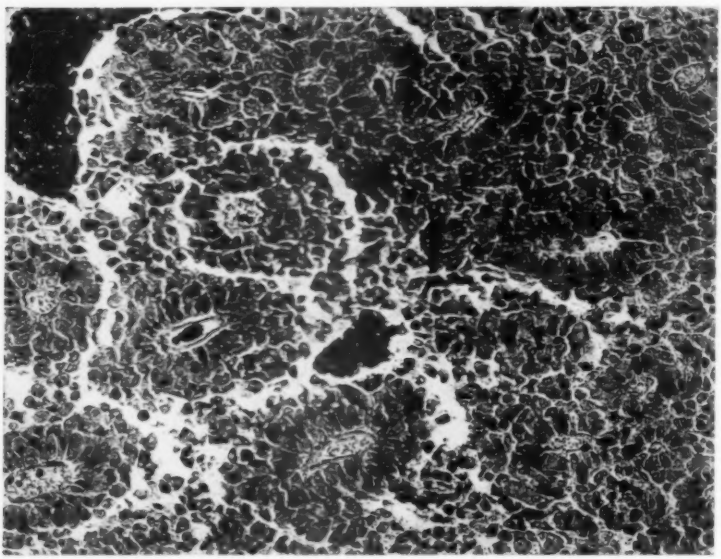

FIG. 3. SECTION OF THE ADENOMA SHOWING THE FORM OF THE TUMOR CELLS AND THEIR RELATION TO THE SMALL BLOOD VESSELS

which have ordinarily been used for this purpose, these cells are found to contain fine blue-staining granules in rather small numbers, most conspicuous in the best preserved cells about the tiny vessels, and there the granules are found at the end of the cell opposite to that which comes into contact with the vessel. This is characteristic of the rather columnar cells of the pars intermedia, especially where they form the lining of the colloid-containing acini. This description

could not apply to the basophilic cells of the anterior lobe proper, for they are rounded or ovoid cells with relatively large nucleus, and with the stains just mentioned show a great quantity of rather coarse, deeply staining, granules filling closely the whole cytoplasm. They stand in no particular relation to the capillaries but seem scattered in a rather haphazard way with eosinophile cells or with both eosinophiles and chromophobes in the small irregular groupings (Fig. 4).

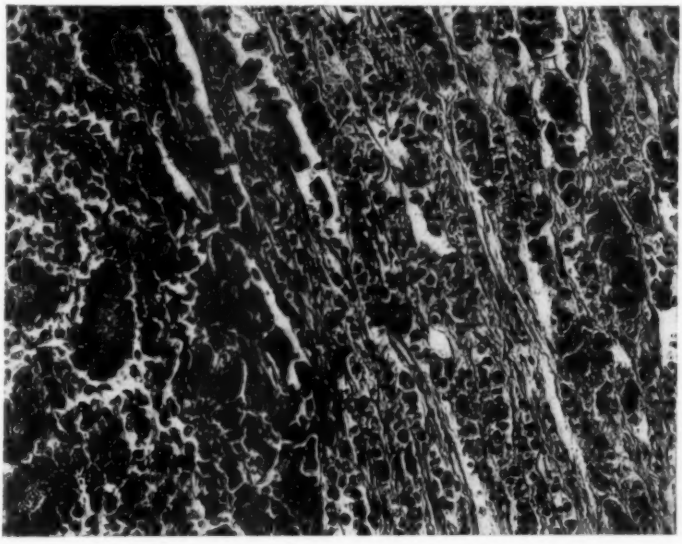

FIG. 4. MARGIN OF THE TUMOR (MANN'S STAIN) SHOWING BASOPHILIC CELLS IN THE COMPRESSED TISSUE OF THE ADJACENT ANTERIOR LOBE

Nevertheless, the fact that the basophiles of the anterior lobe and most of the cells of the pars intermedia including those which are so generally found spread into the substance of the posterior lobe, stain blue with the usual differential stains, and that the adenoma associated with the symptoms described also stains blue or bluish has established the designation *basophile adenoma* for such tumors. Erdheim (4) described such an adenoma in 1910, recognizing its basophilic character by the use of kresofuchsin which stains the baso-

philic cells of the anterior lobe and distinguishing it from an eosinophile adenoma which stains like the eosinophile cells with Heidenhain's iron-haematoxylin. Neither of these stains is of service for any further analysis of the relation of the tumor to other cells, nor is the connective tissue stain of Mallory nor the trichrome stain of Masson, since they stain all these cells alike.

However, it was found that a copper haematoxylin stain,[1] practically as described by Cowdry, offers a more specific method of differentiation since, when applied to tissue fixed in Zenker-formol, it stains the basophile cells of the anterior lobe black but does not stain the cells of the pars intermedia—and does not stain the cells of the tumor (Fig. 5). This seems to afford strong support for the idea that the tumor, merging as it does into the intermediate zone and sharply outlined against the anterior lobe which it compresses, composed of cells morphologically like those of the intermediate zone and different from the basophile cells of the anterior lobe and now staining or rather remaining unstained with those of the intermediate zone while the basophiles of the anterior lobe are sharply and deeply stained, is really derived from the tissue of the pars intermedia.

This idea is further supported to some extent by the work of Holmquist (5) and of Jores and Beck (6), who find that extracts of the intermediate lobe (intermedin or the melanophore hormone) increase the bulk of the adrenal cortex, and by that of Jores and Glogener (7), who found the melanophore hormone in the extract from a basophile adenoma.

The contrast between the unstained pars intermedia and tumor, and the deeply stained basophile cells of the anterior lobe is obvious at once but it remains necessary to determine with certainty that the

[1] The copper haematoxylin stain is not satisfactory after formalin fixation and in any case it seems to require a relatively fresh solution of haematoxylin. The sections, after removal of paraffin and passage through iodine and hyposulphite solutions to remove the mercury precipitate, are put in a saturated solution of copper acetate, washed in distilled water, transferred to a weak haematoxylin solution made by adding 2 cc. of a well ripened 10 per cent solution of haematoxylin in absolute alcohol to 50 cc. of distilled water. From this, after washing, to a 3 per cent solution of bichromate of potassium after which the blackened section is differentiated in Weigert's borax ferricyanide mixture until the color is a deep grayish yellow.

cells which stain in the anterior lobe round about the unstained tumor really are the basophile cells which stain blue with the Mann's methyl-blue-eosin stain. For this purpose it became essential to stain a section with Mann's stain and after careful differentiation to mark out an area in which eosinophile, basophile and chromophobe, were very distinctly grouped in contrast with each other. Such an area

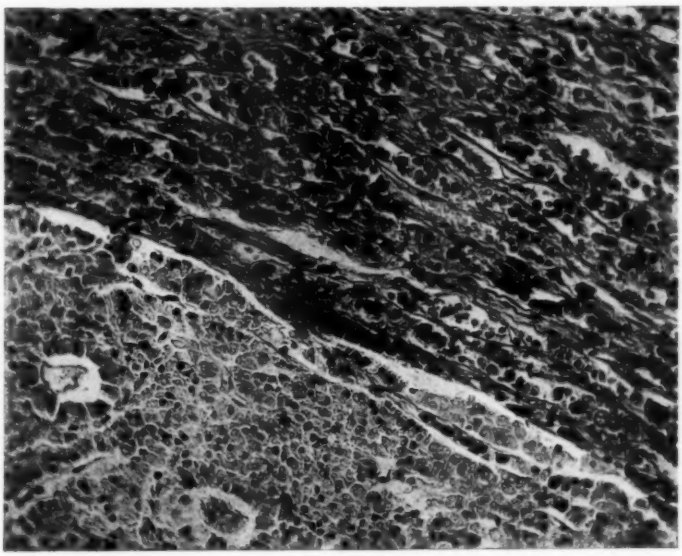

FIG. 5. MARGIN OF THE TUMOR (COPPER HAEMATOXYLIN) SHOWING THE UNSTAINED TUMOR CELLS CONTRASTING WITH THE BASOPHILES OF THE ANTERIOR LOBE

indicated by a diamond scratch on the under side of the slide was photographed with rather high magnification and the coverslip then removed, the section decolorised and restained in copper haematoxylin and again photographed to show the same cells with a new stain. For convenience we have again removed the coverslip, decolorised in permanganate and oxalic and restained with Mann's stain. Then, with the same field under the microscope and the two photographs side

by side under a pane of glass, the individual cells can be compared. This has been done again and again with normal hypophyses.

The basophile cells stain distinctly with the blue-black granules brought out by the copper haematoxylin and correspond faithfully in section and photographs. But there are also some, but not all, of the eosinophiles which take the stain—not whole-heartedly like the basophiles but partially or sometimes completely. It is impossible to say why some stain and others do not, but it suggests the possibility that there may be more types of cell than the three so generally recognised. The chromophobes do not stain, and, as has been said and as the photographs show, the cells of the adenoma and those of the pars intermedia do not stain.

It is hoped that some other stain may be found which will react positively, and not negatively, with the cells of the adenoma and of the pars intermedia and still differentiate them from the basophile cells of the anterior lobe.

If we admit that such an adenoma is derived from the cells of the pars intermedia, a conclusion from which there seems no escape, it becomes necessary to attempt some explanation of the profound disturbances in function which accompany it in so characteristic a manner. These may be thought of as (A) due to some product of the tumor cells, perhaps as an accentuation of the functional activity of the pars intermedia, or (B) as the result of compression of the cells of the anterior lobe interfering with their function. It is even conceivable that interference with the function of the posterior lobe might be considered, although there is no anatomical evidence of pressure as there is in the anterior lobe. But there are many cases in which chromophobe or even eosinophile adenomata cause far more marked compression of the anterior lobe without producing these disturbances.

In the present state of our knowledge it is impossible to offer any satisfactory explanation of any of these phenomena. Heretofore attention has been concentrated in the case of some investigators upon the posterior lobe, of others upon the anterior lobe, and little or nothing has been done in connection with the functions of the pars intermedia although in most animals it occupies such a prominent position. And it is particularly true that the extreme difficulty experienced in separating the tissues leaves us in doubt as to whether

an extract of the anterior lobe does or does not include an extract of attached pars intermedia—an objection which applies equally to extracts of the posterior lobe, since the cells of the pars intermedia extend so abundantly into its substance in many cases.

It will be even more difficult to obtain, in pure form for implantation or for the making of extracts, the tissue of the pars intermedia. But it seems to be a matter of the utmost importance that more should be learned of the function of this tissue and that it should be kept constantly in mind in endeavouring to explain the symptoms of hypophyseal disease.

As for the Cushing syndrome, it appears that we might suggest that in the light of the work of Anselmino and Hoffman, who demonstrate a ketogenic or fat metabolism hormone in extracts of the anterior lobe, it could be assumed that, since in this case no acetone bodies were found in blood or urine and great quantities of fat are merely stored in the tissues, the fat metabolism hormone is in abeyance.

To explain the osteoporosis the idea occurs that the parathyroids may be stirred to greater activity and, indeed, the blood calcium at 11.2 mgm. per cent was in this case rather high.

Cushing (9) has suggested, as explanation of the hypertension, an increased activity of the cells of the intermediate zone which so often invade the posterior lobe. Dean Lewis and others produced an extract of the pars intermedia which had a hypertensive action, while Zondek and Krohn found no such effect from their extract, intermedin, its sole activity being exerted upon the melanophore cells. More recently several authors have found that intermedin has an antidiuretic influence and can be used with success in combating diabetes insipidus although less effective than pituitrin.

The diabetes, with polyphagia and polydipsia and polyuria, brings up a still more difficult problem. A similar refractory diabetes, not easily influenced by insulin, may occur in acromegalics in whom the adenoma is eosinophile with similar compression of the surrounding cells of the anterior lobe. Are we to believe that either type of adenoma produces an excess of Houssay's hormone antagonising the insulin, or that the effect of the adenoma is to stimulate the out-pouring of adrenaline from the adrenal medulla through its action by way of the sympathetic system so as to produce an excess of blood sugar

from the liver glycogen? Or is it possible that the influence of this adenoma upon the adrenal cortex, or at least the similarity of its effects to those of a cortical adrenal tumor, may suggest the much disputed effect of the adrenal cortex upon sugar metabolism?

The gradual cessation of menstruation is consistent with the complete absence of gonad stimulating hormones in the urine, as demonstrated in this case and in others. This is true also of the cases of tumors arising from the adrenal cortex in which sexual alterations are observed, although Frank (8) has found large amounts of folliculin or theelin in the urine in such cases.

Why the gonadal stimulating activity of the hypophysis is lost is not clear. The idea so often expressed that the basophile cells of the anterior lobe are responsible for the production of the gonad stimulating hormones rests on no very sound basis, but since the adenoma appears to be derived from the cells of the pars intermedia, one is at least relieved of what seemed a contradictory result of the increase of basophile cells.

All of these problems have been discussed by Cushing in his Harvey Lecture without satisfactory conclusions in the case of any of them and it seems that they remain for further study which is urgently needed.

CONCLUSIONS

A typical case of the Cushing syndrome is described with symptoms and anatomical changes corresponding with those already known.

The cells of the tumor are cylindrical and arranged radially about capillary blood vessels. In their form they resemble the cells of the pars intermedia and are different in form and arrangement from the basophile cells of the anterior lobe. Further, upon staining with copper haematoxylin it is found that, while the basophiles of the anterior lobe are stained black, the cells of the tumor, like those of the pars intermedia, including those which radiate into the posterior lobe, remain unstained. For these reasons and because of its continuity with that tissue, it is concluded that the tumor is derived from pars intermedia cells.

If this be true, the remarkable disturbances of function which accompany the presence of this tumor, most of which have been

thought dependent upon some change in anterior or posterior lobe, may rouse new interest in the activities of the pars intermedia.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

System of Diet Writing. By WILLIAM S. COLLENS. [Loose-leaf Booklet.] Including: Diet Calculator, Obesity Chart, Diet Formulary and 100 Menu Prescription Forms. 1934. (*Form Publishing Co., New York.*)

Dr. Collens presents a comprehensive outline of the working points of dietetics. He covers the field thoroughly in each case, defining the diet, listing the types of food permitted and stating in what conditions this or that diet is indicated. By means of an ingenious diet calculator in the form of dials and windows, the mathematics of the diabetic menu has been almost entirely eliminated. Likewise, for the diet in obesity and the ketogenic diet in epilepsy, Dr. Collens offers a slide rule which can be readily adjusted to give the amounts of different foods allowed for the desired protein, fat, carbohydrate and calories. The measurements on these calculators are given accurately in terms of grams and cubic centimeters which can be converted into household measures by consulting appropriate tables. For the most part the servings as given by these calculators are normal. It is unavoidable even in such a detailed system that at times for certain foods the amount of the individual serving will either exceed or fall short of the average.

However, Dr. Collens has succeeded in giving fairly practical, pliable tables with a wealth of specific information which should accomplish his purpose to help the doctor bridge the "gap" between his "metabolic research" and his "medical practice."

H. B.

Essentials of Infant Feeding and Paediatric Practice. By HENRY P. WRIGHT. 1934. 212 pp. (*Oxford University Press, New York.*)

This volume starts out admirably. The first half is a good presentation of infant feeding. From there on, however, the author seems to have lost interest, for the last half is hardly more than an outline of the treatment of a few of the conditions met with in childhood. The result is a book too technical for mothers and far too fragmentary for students or physicians.

J. C. B.

Brucella Infections in Animals and Man. Methods of Laboratory Diagnosis. By I. FOREST HUDDLESON. 1934. 108 pp. (*The Commonwealth Fund, New York.*)

The absence of pathognomonic physical signs in undulant fever, as it occurs in man, often renders the clinical diagnosis difficult. Consequently, bacteri-

ological and serological procedures assume first importance in establishing the correct diagnosis. "Brucella Infections in Animals and Man" by I. Forest Huddleson is a monograph giving the technical details of acceptable laboratory methods which have proved of value both for hospital practice and also in veterinary medicine. The clear description of detailed technique makes the book a laboratory manual of practical usefulness. There are photographic illustrations and a bibliography of 188 references.

W. S. T.

A Textbook of Pathology. An Introduction to Medicine. By WILLIAM BOYD. 1934. 2nd edition. $10.00. (*Lea & Febiger, Philadelphia.*)

This new edition of Boyd's well-known text has undergone many alterations. The book is excellent from the standpoint of the medical student and practitioner, for it presents pathology from the clinical side. It is succinct and well-written.

A. G.

Tuberculosis in the Child and the Adult. By FRANCIS MARION POTTENGER. 1934. With 85 illust. in the text. pp. iii plus 611. $8.50. (*C. V. Mosby Co., St. Louis, Mo.*)

Dr. Pottenger dedicates this book to "an enlightened medical profession," and has attempted an ambitious survey of a large subject. It covers, according to the title-page, a discussion of pathological anatomy, pathological physiology, immunology, diagnosis and treatment.

One gains something from reading the opinions of a distinguished clinician as to the rôles of allergy, resistance, immunity, and their problematical interrelationships, but Dr. Pottenger adds nothing new, and this confused field suffers as usual because of our inexact knowledge and the vagueness of our definitions.

The author has a clear idea of the problem of childhood tuberculosis and the relatively benign outcome once infancy is past. He supports his impressions by no new statistics, but there are very few good data on morbidity and mortality available. The clinical chapters and therapeutic suggestions with reference to adult disease are the ones which will be most helpful and best understood by the general practitioner. The chapters on x-ray and on the newer surgical procedures are disappointingly brief and inadequate.

Dr. Pottenger writes interestingly two long chapters on the visceral neurology of pulmonary tuberculosis. This is his own unique contribution and represents an aspect of the disease which most clinicians overlook. It would be of interest if he would write in similar detail of his long experience in tuberculin therapy. Of this he gives a general discussion but without specific data in support of his thesis that such therapy is helpful, and without instructions as to its use.

My impression is that this one is as good as most survey books of a large chaotic medical field. There are chapters which will well repay reading. The bibliography covers most of the important contributions, but several errors are to be found in the foreign references.

M. B.

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THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore, Maryland

Made in United States of America

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OF
THE JOHNS HOPKINS HOSPITAL

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This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

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BALTIMORE, MARYLAND, U. S. A.

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